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EVIDENCE FOR THE GENERATION OF A \textit{m}-NAPHTHYNE

by

Dorothy Wolf

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

Thesis Director's Signature:

\underline{W.S. Billsop}  
April, 1975
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Finally, without the encouragement of my husband and my parents I would not have persevered through the past four years.
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EVIDENCE FOR THE GENERATION OF A m-NAPHTHYNE
INTRODUCTION

Although the chemical and physical properties of \( o \)-benzyne \( \mathbf{1} \) have been well enough investigated to accept it as a reactive intermediate, the \textit{meta} \( \mathbf{2} \) and \textit{para} \( \mathbf{3} \) isomers have not gained such recognition.

\[ \begin{align*}
\text{\( o \)-benzyne} & \quad \text{\( m \)-benzyne} & \quad \text{\( p \)-benzyne} \\
\mathbf{1} & \quad \mathbf{2} & \quad \mathbf{3}
\end{align*} \]

The existence of \( o \)-benzyne \( \mathbf{1} \) was accepted when Roberts, Semenov, Simmons and Carlsmith\(^1\) reacted \(^{14}\)carbon labelled bromo-benzene \( \mathbf{4} \) with potassium amide to produce aniline \( \mathbf{5} \) labelled in the positions.

\[ \text{Reaction 1} \]

\[ \begin{align*}
\text{\( o \)-bromobenzene} & \quad \text{\( KNH_2 \)} \\
\mathbf{4} & \quad \xrightarrow{\text{\( KNH_2 \)}} \quad \mathbf{5} \quad \mathbf{5} \quad \text{\( NH_2 \)}
\end{align*} \]

one and two positions. Since then \( o \)-benzyne \( \mathbf{1} \) has been well studied in its reactions toward nucleophiles and dienes.\(^2\) Recently \( o \)-benzyne \( \mathbf{1} \) was isolated in a matrix and its infrared spectrum recorded.\(^3\)

The \textit{para} isomer \( \mathbf{3} \) was first postulated in the thermolysis of \( 1,4 \)-diiodobenzene\(^4\) and the photolysis of \( 4 \)-benzenediazonium carboxylate.\(^5\) These decompositions yielded a mass spectral peak.
at m/e 76 with an ionization potential of 9.46 eV. Bergman\textsuperscript{6} characterized the \textsuperscript{\textup{m}}-benzyne \( \mathcal{Z} \) as a diradical \( \mathcal{Z}_c \) when he trapped the intermediate in the rearrangement of \( 1,6\)-dideuterohexa-3-ene-1,5-diyne \( \mathcal{Z} \). Using a hydrocarbon solvent he obtained benzene \( \mathcal{Z} \). Reaction 2

\[
\begin{array}{c}
\text{D} \quad \equiv \quad -\left[ \cdot \right] \quad \equiv \quad \text{D} \\
\text{D} \quad \equiv \quad \text{H} \quad \equiv \quad \text{H} \quad \equiv \quad \text{H} \\
\text{D} \quad \equiv \quad \text{H} \quad \equiv \quad \text{H} \\
\mathcal{Z} \quad \equiv \quad \mathcal{Z}_c \quad \equiv \quad \mathcal{Z} \\
\end{array}
\]

using carbon tetrachloride he obtained 1,4-dichlorobenzene \( \mathcal{Z}_c \) and using methanol he obtained benzyl alcohol \( \mathcal{Z} \). The multiplicity of the diradical \( \mathcal{Z}_c \) is not yet known, however the heat of formation has been estimated as 140 kcal/mole.

The majority of interest in \( \text{m} \)-benzyne \( \mathcal{Z} \) to date has been expressed by the theoretical chemist. Theoreticians seem to be unsure which of the structures, \( \mathcal{Z}_a, \mathcal{Z}_b \) or \( \mathcal{Z}_d \) represents the ground state. In 1967 Evleth\textsuperscript{7} performed a Pariser-Pople-Parr calculation assuming benzene geometry. He determined the total
electron energy would be -81.9463 eV. (Benzene was calculated as
-81.2208 eV.) He also predicted the ionization potential, dipole
moment, electron densities, bond orders and electronic transitions.
A significant prediction was that $m$-benzyne $2_g$ would not have the
charge separated structure $2_g$. The interactions of radical lobes

\[ 2_g \]

in the same molecule were studied in 1968 by Hoffmann, Imamura and
Hehre. This led to the prediction that $2_g$ should be more stable
than the diradical $2_b$. Whereas a Hückel calculation predicts the
$p$-benzyne $2$ to be more stable than the $m$-benzyne $2$ Olsen in 1971,
using the INDO method predicted the opposite. He determined that
$\sigma$-benzyne $2$ has a singlet ground state, whereas $meta 2$ and $para 2$
have ground state triplets approximately 10 kcal/mole below the
singlet state. An ab initio SCF and SCF-CI calculation by Wilhite
and Whitten also in 1971 came to the same conclusion as Olsen.
However, they found a small singlet-triplet splitting for the $meta 2$
and $para 2$ isomers. Finally in 1971, Hess and Schaad determined
the resonance energy per $\pi$ electron (REPE) in units of $\beta$ for
several hydrocarbons. They state that if the value is negative the
hydrocarbon is anti-aromatic, if it is zero it is a polyolefin and
if it is positive it is aromatic. For $m$-benzyne $2$ they calculated
0.055 $\beta$, compared with 0.065 $\beta$ for benzene $2$. The only calculation
to date that has attempted to optimize the geometry of $m$-benzyne $2$
with respect to energy was published in 1974 by Dewar and Li.
Their prediction is a ground state singlet with a heat of formation
<table>
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<td>REPE</td>
<td>$3a \Delta H, 0.055 \beta$</td>
<td>$0.065 \beta$, $-0.268 \beta$, $0.02 \beta$, $-0.002 \beta$</td>
<td>BA Hess, Jr., &amp; LK Schaad, <em>Tetrahedron Lett.</em> 17 (1977).</td>
</tr>
<tr>
<td>MINDO/3</td>
<td>$2a \Delta H, 117.6$, $2b \Delta H, 125.9$</td>
<td>$1a \Delta H, 118.3$, $1b \Delta H, 126.9$, $2a \Delta H, 124.9$, $2b \Delta H, 122.8$, $3a \Delta H, 123.1$, $3b \Delta H, 114.3$, $3c \Delta H, 116.9$, $3d \Delta H, 118.5$</td>
<td>MAJ Dewar &amp; WK Li, <em>J. Amer. Chem. Soc.</em> 98, 5560 (1976).</td>
</tr>
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<td>MINDO/3-CI</td>
<td>$2a \Delta H, 107.2$</td>
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<tr>
<td>Experimental</td>
<td></td>
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</tbody>
</table>
1 to 11 kcal/mole lower than \( \sigma \)-benzyne \( \perp \).

Experimentally the evidence for the existence of \( m \)-benzyne \( \perp \) stems from the studies of the decomposition of benzenediazonium-3-carboxylates \( \perp \). In 1965 Berry, Claridy and Schafer \( \perp \) studied the flash initiated decomposition using optical and mass spectrometry. The mass spectrum showed a parent ion at m/e 76 with a gas phase lifetime comparable to \( \sigma \)-benzyne \( \perp \). Comparison of the optical

Reaction 3
spectrum with diacetylenes and vinyl acetylenes led them to rule out such structures, although at longer times an optical spectrum similar to a vinyl diacetylene was obtained. The isolated products of the reaction were benzene and phenol. The thermolysis of benzenediazonium-3-carboxylates was studied by Bertorello, Rossi and de Rossi. From graphical analysis of the percent carbon dioxide evolved or the pressure of hydrogen chloride applied versus the yield of chlorobenzene and chlorobenzoic acids, they concluded that a zwitterionic $m$-benzyne is an intermediate. Finally, McGriff devoted his dissertation to synthetic approaches to $m$-benzyne via bicyclo[3.1.0]hexanes. Besides identifying several substituted bicyclo[3.1.0]hexanes, he produced an intractable polymer when heating 2,3-diacetoxy-6-$N,N$-dimethylnitroxide bicyclo[3.1.0]hexane. Simultaneously with the investigation of benzyne chemistry
has been the investigation of heteraryne chemistry - especially nitrogen heterocycles. In a theoretical investigation of the Chichibabin reaction Jones and Beveridge\textsuperscript{16} studied the nitrogen lone pair interaction with a 2,3-pyridyne\textsuperscript{12}. Their conclusion was that if a molecular orbital were formed, the lowest electron density would fall on the C2 carbon, yielding the observed 2-aminopyridine\textsuperscript{13}.

Reaction 5

\[
\text{N} \quad \text{NH}_2^- \quad \text{NH}_3 \quad \text{H}_2\text{N}\]

They further suggested that 2,6-pyridyne\textsuperscript{14} is even more stable, however it is not formed since the 3 position is more favorable for hydride elimination than the 6 position. In the case of isoquinoline\textsuperscript{15}

Reaction 6

\[
\text{N} \quad \text{NH}_2^- \quad \text{NH}_3 \quad \text{NH}_2\]

the most acidic proton is in the one position and the three position is most favorable for hydride elimination. Jones and Beveridge do postulate a 1,3-isoquinolyno\textsuperscript{16} in this Chichibabin reaction.

Further evidence for a 2,6-pyridyne\textsuperscript{14} was suggested by Kauffmann and Boettcher\textsuperscript{17} when they demonstrated that 2-chloro-
2-bromo-, and 2-iodopyridine in reaction with lithium piperidide, lithium cyclohexylamide or lithium diethylamide resulted in a resin and lithium halide. When a methyl group was substituted in the six position no resin was formed. Van der Lans, den Hertog and van Veldhuizen repeated these experiments at -60^\circ C and obtained the ring opened compound 17 which they believe polymerized as Kauffmann and Boettcher observed.

In view of the theoreticians predictions that m-benzylene 17 should be as stable as o-benzylene 15, it is quite surprising that organic chemists have not considered it as an intermediate, especially in certain reactions and observations that are difficult to explain via an o-benzylene 15.

First, the preferred direction of addition to an o-benzylene 17 is predicted on the basis of electronic inductive effects. Yet, despite opposite electronic effects, m-halo-\(\alpha\)-trifluorotoluenes and m-haloanisole produce only the three substituted anilides. Furthermore,
Roberts, Vaughan, Carlsmith and Semenow\textsuperscript{20} point out that attack of the nucleophile on the "triple bond" probably takes place in the plane of the ring and therefore should have little effect on the electron cloud which transmits those inductive effects. This makes doubtful the application to benzyne chemistry of electronic theory as it applies to, for example, aromatic electrophilic substitution.

Secondly, Gilman and Gorsich\textsuperscript{21} invoked abstraction by n-butyl lithium of a bromonium ion from o-bromohalobenzenes\textsuperscript{18} to account for the formation of only o-halobenzoic acids\textsuperscript{19} on the addition of carbon dioxide to the reaction. However, the work by Gilman, Crouse, Massie, Benkeser and Spatz\textsuperscript{22} refute this idea.

1-Bromonaphthalene\textsuperscript{20} on treatment with lithium diethylamide (which
yields 2-diethylaminonaphthalene\(^{21}\) followed by treatment with carbon dioxide gave no naphthoic acids\(^{22}\). On the other hand, treatment of 1-bromonaphthalene\(^{20}\) with \(\text{\textit{n}}\)-propyl lithium followed by carbon dioxide yields only 1-naphthoic acid\(^{22}\). This has led to much confusion over the assumed "lithium-halogen interconversion" which has only been substantially documented in alkyl cases.

Finally, reductions of halonaphthalenes and halobenzenes have been reported in almost all systems of base and solvent in which \(\text{o}\)-benzyne\(^{14}\) is formed. Table II is a listing of all systems found in the literature in which reduction has been observed. Among the large variety of substrates, bases and solvents, the only apparent common element is that all substrates have at least one \underline{meta} proton. The only exception is 9-bromophenanthrene which has a proton in the \underline{eight} position.

For each system, independent mechanisms of reduction have been proposed but never proven, and often later investigations have shed doubt on them. In the case where \(\text{\textit{t}}\)-butoxide acts as base the following scheme has been proposed:\(^{28}\)

\begin{equation}
\text{\textit{t}}\text{BuO}^- + \text{CH}_3\text{SOCH}_3 \xrightleftharpoons{} \text{\textit{t}}\text{BuOH} + \text{CH}_3\text{SOCH}_2^-
\end{equation}

\begin{equation}
\text{CH}_3\text{SOCH}_2^- + \text{ArX} \rightarrow \text{Ar}^- + \text{CH}_3\text{SOCH}_2\text{X}
\end{equation}

\begin{equation}
\text{Ar}^- + \text{\textit{t}}\text{BuOH} \rightarrow \text{ArH} + \text{\textit{t}}\text{BuO}^-
\end{equation}

\begin{equation}
\text{CH}_3\text{SOCH}_2\text{X} + \text{Base} \rightarrow \text{X}^- + ?
\end{equation}

When Bradshaw and Hales\(^{30}\) observed reduction, using this solvent and base, in their studies of 1- and 2-halonaphthalenes, they independently reacted the halonaphthalenes with dimethyl sodium. Far lower yields of
<table>
<thead>
<tr>
<th>No.</th>
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<th>Base</th>
<th>Solvent</th>
<th>Reduction δᵣ</th>
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<td>23</td>
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<td>Li N(C₆H₄)₂</td>
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<td>10</td>
<td>23</td>
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<tr>
<td>3</td>
<td><img src="image3" alt="Substrate 3" /></td>
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<td>Ether</td>
<td>15</td>
<td>23</td>
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<td>4</td>
<td><img src="image4" alt="Substrate 4" /></td>
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<td>32</td>
<td>23</td>
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<td><img src="image5" alt="Substrate 5" /></td>
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<td>23</td>
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<td><img src="image7" alt="Substrate 7" /></td>
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<td>8</td>
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<td><img src="image12" alt="Substrate 12" /></td>
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<td>KOtBu</td>
<td>tBuOH/DMF</td>
<td>0.1</td>
<td>28</td>
</tr>
<tr>
<td>37</td>
<td>![Substrate Image]</td>
<td>KOtBu</td>
<td>tBuOH/HMPA</td>
<td>0.08</td>
<td>28</td>
</tr>
<tr>
<td>39</td>
<td>![Substrate Image]</td>
<td>KOtBu</td>
<td>DMSO</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>40</td>
<td>![Substrate Image]</td>
<td>Dimethyl Sodium</td>
<td>DMSO</td>
<td>1.3</td>
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<tr>
<td>41</td>
<td>![Substrate Image]</td>
<td>LiPip</td>
<td>Piperidine</td>
<td>2.6</td>
<td>31</td>
</tr>
<tr>
<td>42</td>
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<td>KOtBu</td>
<td>DMSO</td>
<td>1.0</td>
<td>29</td>
</tr>
<tr>
<td>43</td>
<td>![Substrate Image]</td>
<td>KOtBu</td>
<td>DMSO</td>
<td>4.6</td>
<td>29</td>
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<tr>
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<td>![Substrate Image]</td>
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<td>Piperidine</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>45</td>
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<td>DMSO</td>
<td>2.2</td>
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<td>6.5</td>
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<td>47</td>
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<td>4.2</td>
<td>29</td>
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<tr>
<td>49</td>
<td>![Substrate Image]</td>
<td>LiPip</td>
<td>Piperidine</td>
<td>14</td>
<td>31</td>
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<tr>
<td>50</td>
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<td>Piperidine</td>
<td>31</td>
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<tr>
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<td>LiPip</td>
<td>Ether</td>
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<td>33</td>
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<td>LINCH&lt;sub&gt;3&lt;/sub&gt;P</td>
<td>Ether</td>
<td>20</td>
<td>34</td>
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<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10-15</td>
<td>35</td>
</tr>
<tr>
<td>54</td>
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<td>KNH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5-10</td>
<td>35</td>
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<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1</td>
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<tr>
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<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>25</td>
<td>35</td>
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</tbody>
</table>

<sup>a</sup> Halogen reduced

<sup>b</sup> Rate relative to entry
reduced product were observed. Benkeser and DeBoer\textsuperscript{23} proposed a six-membered transition state\textsuperscript{22} in the reduction using lithium amides.

\textbf{Reaction 12}

\[
\begin{align*}
\text{OCH}_3 & \quad \xrightarrow{\text{Br}} \quad \text{LiNR'}(\text{CH}_2\text{R}) \\
\text{C}_6\text{H}_5 & \quad \xrightarrow{\text{LiNR'}} \quad \text{CH}_3\text{O} \\
\end{align*}
\]

Since this is essentially a hydride transfer, they attempted the reduction using lithium aluminum hydride (which in ether is more soluble than lithium hydride). No reduced material was obtained. Their attempts to isolate imines, predicted by the mechanism also

\textbf{Reaction 13}

\[
\begin{align*}
\text{X} & \quad \xrightarrow{\text{LiNR'}(\text{CH}_2\text{R})} \quad \text{C}_6\text{H}_5 \quad + \quad \text{LiX} \quad + \quad \text{R'} \quad \text{HNCH}_2\text{R} \\
& \quad \xrightarrow{\text{LiNR'}(\text{CH}_2\text{R})} \quad \text{C}_6\text{H}_5 \quad \xrightarrow{\text{LiNR'}(\text{CH}_2\text{R})} \quad \text{LiC}_6\text{H}_5 \\
& \quad \xrightarrow{\text{RCH=NR'}} \quad \text{RCH=NR'} \\
& \quad \text{CH}_3\text{O} \quad + \quad \text{LiBr} \\
\end{align*}
\]
failed. However, in some instances they isolated pyrazines which they claimed may be oxidized imines. They did not further check whether the pyrazines were possibly formed without substrate present. Finally, Wittig, Schmidt and Renner proposed o-benzyne leading to a phenyl lithium as the reducing mechanism. This however, requires at least one ortho hydrogen atom, which some substrates do not have. It also requires the formation of an imine.

Several seemingly unusual reactions also exist in the heteraryne field. Den Hertog, Pieterse and Buurman reported several reactions which could go through 1,3 eliminations — although their explanations are o-pyridynes followed by inductive direction of the entering amide. Postulation of o-pyridynes from 2-halo pyridines is unexpected, since the 3 proton, which must be abstracted to form the 2,3-pyridyne, is least acidic. Furthermore, the "unusual meta rearrangement" cannot be explained. The most unusual reactions are surely the rearrangement of 2,6-dihalo pyridines to pyrimidines and the rearrangement of 2-halo quinolines to quinazolines in the presence of potassium amide, observed by den Hertog, van der Plas,
Pieterse and Streef. The following mechanism is proposed for these rearrangements. Treatment of substituted 2-halopyridines with potassium amide in liquid ammonia or lithium piperidide in piperidine at 20-35°C also yielded rearrangements of the pyridine nucleus. Although van der Lans, den Hertog and van Veldhuizen do not propose a mechanism for these rearrangements, the suggestion is strong that 2,6-pyridynes participate.
In order to provide more evidence for the existence of 1,3-arynes, several new bicyclo[3.1.0]hexane systems were synthesized and characterized. It was expected that treatment of \[31, 32, \text{and} \] \[33\] with strong non-nucleophilic bases would eliminate hydrogen halide,
producing the \( m \)-benzyne \( \mathcal{Z} \) or \( m \)-naphthyne \( \mathcal{Y} \). Formation of reduced products would indicate that the \( m \)-aryne behaves as a diradical (such as \( \mathcal{Z}_5 \)) and isotopic labelling of the one and three positions would lend further evidence for the generation of a \( m \)-aryne.
RESULTS

3-Bromo-6,6-dichlorobicyclo[3.1.0]hexane 31 was synthesized in three steps from cyclopentadiene 36. Repetition of the Bartlett and Rice 38 procedure for the synthesis of 4-bromocyclopentene 38

Reaction 18

occurred uneventfully in our laboratory although Johnson and Keiser 39 reported several explosions in the lithium aluminum hydride reduction of 3,5-dibromocyclopentene 37. It was only after repeated distillation that the 4-bromocyclopentene 38 was sufficiently pure to proceed with the carbene addition.

Although micellar carbene additions generally give a higher yield of the dichlorocyclopropyl adduct in this case both the micellar and the standard (potassium t-butoxide and chloroform in pentane) 40 procedures gave only a 7% yield. The structure of the adduct was assigned from its proton magnetic resonance and infrared spectra. No attempt was made to separate the endo and exo isomers and the spectra are possibly slightly more complex than expected for this reason.
Reaction of 3-bromo-6,6-dichlorobicyclo[3.1.0]hexane 31 with potassium t-butoxide in either dimethyl sulfoxide or tetrahydrofuran produced three products. The major one, 98.4% was chlorobenzene 39.

Reaction 19

\[
\begin{align*}
\ce{Br} & \xrightarrow{\text{KOTBu}} \ce{Cl} \\
\ce{Cl} & + \ce{40} + \ce{41} + \text{Tar}
\end{align*}
\]

Gas chromatography on a 20% diocyl phthalate column (specifically used to separate benzene compounds) showed that none of the trace byproducts had the retention time of benzene.

In a totally different, but related system the reaction of bromobenzene 4 with sodium amide in liquid ammonia was repeated. It

Reaction 20

\[
\begin{align*}
\ce{Br} & \xrightarrow{\text{NaNH}_2} \ce{NH}_2 \\
\text{liq NH}_3 & + \ce{5} + \ce{7} + \text{Tar}
\end{align*}
\]

was found that a 2% yield of reduced material - benzene 7 (identified by its boiling point, ultraviolet spectrum and gas chromatography retention times) was obtained in addition to the aniline 5 (16% yield) reported by Roberts and coworkers. 1

The synthesis of 2,3-benzo-6,6-dichlorobicyclo[3.1.0]hexane 32 in 57% yield from indene 42 via a micellar carbene addition is a clear example of the improved yield over more conventional methods, i.e. potassium t-butoxide and chloroform in pentane. Parham, Reiff and
Reaction 21

\[
\text{苯} \xrightarrow{\text{Micellar :CCl}_2} \text{氯代芳香环}
\]

Swartzentruber\textsuperscript{41} first isolated this compound in 2-4\% yield using indene itself as the solvent and potassium tert-butoxide as the base.

Besides the properties of 2,3-benzo-6,6-dichlorobicyclo[3.1.0]hexane\textsuperscript{32} (mp: 74-76°C) matching Parham's compound, its structure was confirmed by the spectral data. In the mass spectrum, it displayed a weak parent ion at m/e 198 with the intensities of the 200 and 202 peaks establishing the presence of two chlorine atoms.

The base peak was at 163 (loss of Cl), with further strong peaks at 127 (loss of HCl and Cl) and 128 (loss of Cl\textsubscript{2}). The PMR spectrum showed a one proton multiplet at 62.42 ppm, a two proton peak at 63.09 ppm overlapping with a one proton multiplet at 63.19 ppm and a four proton aromatic region at 66.97 ppm. The \textsuperscript{13}C carbon magnetic resonance spectrum showed ten carbon atoms of which four were sp\textsuperscript{3} hybridized and six were aromatic. The proton decoupled spectrum gave the sp\textsuperscript{3} signals at 34.09 ppm, 35.44 ppm, 42.90 ppm and a quaternary (R\textsubscript{2}CCl\textsubscript{2}) at 66.10 ppm. The four non-quaternary aromatic signals could easily be seen at 124.22 ppm, 125.09 ppm, 126.30 ppm and 127.42 ppm; and the quaternary aromatic signals, with their narrower linewidth, could be distinguished at 139.43 ppm and 143.83 ppm. The non-decoupled spectrum showed the signal at 34.09 ppm to be a triplet (J \approx 2.0 ppm); the signal at 35.44 ppm to be a doublet (J \approx 1.6 ppm); and the signal at 42.90 ppm to be a doublet (J \approx 2.4 ppm).
In order to synthesize the parent hydrocarbon and to further confirm the carbon skeleton, 2,3-benzo-6,6-dichlorobicyclo[3.1.0]hexane 32 was submitted to a sodium-ammonia reduction. This produced a quantitative conversion to the desired 2,3-benzobicyclo[3.1.0]hexane 43 (45.7% yield) and tetralin 44 (54.3% yield). Gave Reaction 22

\[
\text{Cl} \quad \text{Cl} \quad \text{Na} \quad \text{liq NH}_3 \quad \rightarrow \quad \text{benzene} + \text{tetralin}
\]

an exact mass of 130.0777 (calculated: 130.0782) and showed strong peaks in the infrared due to cyclopropyl methylene stretches (3075, 3050 and 3040 cm\(^{-1}\)) and skeletal vibrations (1025, 1018 and 1002 cm\(^{-1}\)). The PMR spectrum showed a one proton quartet (\(J = 4\) Hz) at 6.02 ppm due to the exo C6 proton, a one proton triplet-of-doublets (\(J = 4\) Hz, \(J = 7.7\) Hz) at 80.98 ppm due to the endo C6 proton, a one proton multiplet at 81.74 ppm due to the C5 proton, another one proton multiplet at 82.28 ppm due to the C1 proton, a two proton multiplet at 82.96 ppm due to the C4 protons and a four proton aromatic region at 86.97 ppm. The Karplus relationship determines that the exo C6 proton should be coupled to the proton at C1 and C5 with a coupling constant of
approximately 4 Hz, which is, by coincidence, the same value as the geminal coupling constant. The endo proton is closer to the aromatic ring and therefore more deshielded than the exo proton. Since the proton at Cl is benzylic it is assigned the downfield signal of the two multiplets.

The 2,3-benzo-6,6-dichlorobicyclo[3.1.0]hexane \( \text{22} \) was reacted with potassium \( t \)-butoxide in either tetrahydrofuran or dimethyl sulfoxide, after determining that the starting material did not solvolyze in these solvents. In tetrahydrofuran the reaction proceeded quite cleanly to give 99.9% 2-chloronaphthalene \( \text{45} \) and 0.1% naphthalene \( \text{46} \). These two products were isolated by preparative gas chromatography (no other products were detected) and identified by their melting points and mass spectra.

Using dimethyl sulfoxide as solvent two additional byproducts

\[ \text{22} \xrightarrow{\text{KOTBu \ THF}} \text{45} \quad \text{46} \]
were isolated by preparative gas chromatography. What appears to be a methoxynaphthalene 48 (parent ion at m/e 158) was isolated in 0.2% yield and what appears to be a naphthol 47 (parent ion at m/e 144) was isolated in 0.4% yield. The yield of naphthalene 46 remained constant, while the yield of 2-chloronaphthalene 45 decreased to 99.2%. An interesting sidelight of this reaction was the appearance of a blue band on the thin layer chromatography plate at Rf approximately 0.11 if the plate was allowed to sit three to four hours before removing the materials from the plate. If the material was removed from the plate, the color change occurred in solution. Parham and Reiff 42 reported an azulene 49 as a byproduct in the reaction of indenyl sodium 50 with chloroform. Several attempts to purify this blue band were unsuccessful.

What appeared to be a simple three step synthesis of 1-bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane 33, proved to be more
difficult than expected. Indene \( \text{52} \) was easily brominated according to the procedure of Winstein and Roberts\(^{43} \) in 81-89% yield. The distilled liquid proved to be a mixture of 83% trans- and 17% cis-dibromoindene \( \text{52} \) as reported by Rosen, Dorfman and Linfield\(^{44} \) in their NMR studies of dibromoindan \( \text{51} \).

The elimination of hydrogen bromide to give only the 2-bromoindene \( \text{52} \) isomer proved far more difficult than the literature indicated. Porter and Suter\(^{45} \) claim to have synthesized pure 2-bromoindene \( \text{52} \) either by heating dibromoindan \( \text{51} \) in vacuo one hour or by pyrolyzing \( \text{51} \) in refluxing tetralin for four hours. In our laboratory gas chromatographic analysis showed that both procedures gave mixtures of three materials in almost equal proportions plus mainly recovered dibromoindan \( \text{51} \). In the search for a reaction that would give only the 2-bromoindene \( \text{52} \) isomer 1) alcoholic potassium hydroxide, 2) triethylamine and 3) lithium chloride in refluxing dimethylformamide were all tried. The first gave five products; the second gave starting material and two products; and the last gave in 60% yield a one-to-one mixture of 2-chloroindene \( \text{53} \) and 2-bromoindene \( \text{52} \).

**Reaction 27**

\[
\text{51} \xrightarrow{\text{LiCl, DMF}} \text{52} + \text{53}
\]

No reaction occurred when lithium bromide was substituted for lithium chloride. However, the addition of 1.6 molar equivalents of lithium carbonate (as described by Corey and Hortman\(^{46} \)) to the lithium bromide in refluxing dimethylformamide gave a 55-58% yield of pure (after
reocrystallization, mp: 38-38.5°C) 2-bromoindene \( \text{52} \) in three to four hours. This reaction was also successful in the hands of Blakeney (this laboratory) in the synthesis of 1,2-dihydro-3-bromonaphthalene \( \text{55} \).

**Reaction 28**

\[
\begin{array}{c}
\text{Br} \\
\text{Br} \\
\downarrow \\
\text{LiBr - Li}_2\text{CO}_3 \\
\text{DMF} \\
\hline
\text{Br}
\end{array}
\quad \text{52} \quad \text{55}
\]

Since Parham and Wright\(^7\) established that dichlorocarbene does not react with materials containing an electron withdrawing group on the double bond, it was important to demonstrate the purity and structure of the 2-bromoindene \( \text{52} \). Thin layer chromatography on silica gel using six solvents with varying dielectric constants, as well as gas chromatography on a Carbowax 20M or an SE-30 column at several temperatures all showed only one compound. The low resolution mass spectrum gave weak parent ions of equal intensity at m/e 194 and 196 as expected of a bromine containing material. The base peak at m/e 115 results from loss of bromine. The PMR spectrum showed a two proton benzylic doublet \((J = 1.6 \text{ Hz})\) at 83.44 ppm, a one proton benzylic and olefinic triplet \((J = 1.6 \text{ Hz})\) at 66.69 ppm and a four proton aromatic region at 87.01 ppm. As expected, the cyclopentadiene part of the system is planar, since no geminal splitting of the benzylic protons is observed (that is, the two C3 benzylic protons are magnetically equivalent).

The micellar carbene addition as described by Joshi, Singh and Pande\(^8\) was attempted on the 2-bromoindene \( \text{52} \). The products isolated were a 19:1 mixture of 2-bromo-3-chloronaphthalene \( \text{56} \) and
2,3-dichloronaphthalene \( \text{57} \). This indicated that the carbene had added. However, at the reaction temperature of 55-60\(^{\circ}\)C the adduct \( \text{33} \) had opened. On repetition of the reaction at room temperature, followed by low temperature recrystallization of the product, a white crystalline material (mp: 80-82\(^{\circ}\)C with decomposition) was obtained. This carbene addition does not seem to be general, since we have not succeeded in
adding dichlorocarbene to 1,2-dihydro-3-bromonaphthalene \textsuperscript{58} or 9-bromophenanthrene \textsuperscript{58}. The low resolution mass spectrum at 70 eV showed no parent ion at m/e 276. However, the base peaks at 162.0227 (C\textsubscript{10} H\textsubscript{7} Cl calculated: 162.0236); 196.9910 (C\textsubscript{10} H\textsubscript{7} Cl\textsubscript{2} calculated: 196.9924); and 242 (exact mass at 239.9334 C\textsubscript{10} H\textsubscript{7} BrCl calculated: 239.9340) established the presence of two chlorine atoms and one bromine atom in the product \textsuperscript{58}.

The PMR spectrum proved to be surprisingly simple. The most important information gained from it was that no proton is present in the Cl position, as indicated by the lack of a peak around 82.42 ppm. What appears to be two singlets at 63.20 ppm (one proton) and 63.71 ppm (two protons) on expansion proves to be a finely divided ABX system. The aromatic region is a four proton complex multiplet around 87.02 ppm.

In order to rule out the possibility of structures such as

\[ \text{Cl} \quad \text{Br} \quad \text{Cl} \quad \text{Cl} \]

\[ \text{H} \quad \text{Cl} \quad \text{Ci} \quad \text{Br} \]

\textsuperscript{58} and \textsuperscript{60} and for comparison with 2,3-benzo-6,6-dichlorobicyclo[3.1.0] hexane \textsuperscript{32} the \textsuperscript{13} carbon magnetic resonance spectrum was obtained. In the proton decoupled spectrum four quaternary carbons, two sp\textsuperscript{3} at 46.19 ppm and 68.33 ppm and two aromatic at 128.07 ppm and 142.62 ppm could easily be detected. Of the remaining six signals two were sp\textsuperscript{3} at 45.90 ppm and 50.02 ppm and four aromatic at 123.59 ppm, 125.09 ppm, 126.74 ppm and 128.00 ppm. The two sp\textsuperscript{3} signals split into a triplet (J \approx 2.2 ppm) and a doublet (J \approx 2.2 ppm) respectively. This
spectrum is entirely consistent with the structure as assigned and
definitely rules out 52 and 60.

As a final confirmation of the carbon skeleton, 1-bromo-3,4-
benzo-6,6-dichlorobicyclo[3.1.0]hexane 33 was submitted to a sodium-
ammonia reduction. A quantitative yield of 16.6% of the hydrocarbon
2,3-benzobicyclo[3.1.0]hexane 43 (spectral data identical with that
obtained previously) and 83.4% 1,4-dihydronaphthalene 61 (spectral data
Reaction 32

\[
\begin{align*}
\text{Cl} & \quad \text{Na} \\
\text{Cl} & \quad \text{liq NH}_3 \\
\text{Br} & \\
\end{align*}
\]

identical with independently synthesized material 49) was obtained.
Rearrangements of the type exhibited in this reaction are rather
uncommon.

The room temperature stability of the 1-bromo-3,4-benzo-6,6-
dichlorobicyclo[3.1.0]hexane 33 in several solvents was checked. After
one hour at room temperature, a solution of 33 in ethyl ether, carbon
tetrachloride, deuterochloroform or tetrahydrofuran showed no changes
in the PMR spectrum. However, in d_6-dimethyl sulfoxide 33 solvolized
with a half-life of 48 minutes at 40°C to a 19:1 mixture of 2-bromo-3-
chloronaphthalene 56 and 2,3-dichloronaphthalene 57. Mass spectral
Reaction 33

\[
\begin{align*}
\text{Cl} & \quad \text{Br} \\
\text{Cl} & \quad \text{DMSO} \\
\text{Br} & \\
\end{align*}
\]
Table III. Product Distributions from Reactions of \( \text{33} \)

<table>
<thead>
<tr>
<th>Products</th>
<th>KOTBu/DMSO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>KOTBu/d&lt;sub&gt;6&lt;/sub&gt;-DMSO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>KOTBu/THF&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Micellar Addition 60&lt;sup&gt;o&lt;/sup&gt;</th>
<th>( \text{33} )&lt;sup&gt;ac&lt;/sup&gt;</th>
<th>Mass Spec</th>
<th>Formula</th>
<th>Melting Point</th>
</tr>
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<td>45</td>
<td>59.2</td>
<td>65.4</td>
<td>19.9</td>
<td></td>
<td></td>
<td>164.0371&lt;sup&gt;d&lt;/sup&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;D&lt;sub&gt;2&lt;/sub&gt;Cl</td>
<td>56-56.5°C</td>
</tr>
<tr>
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<td>164.0359&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;D&lt;sub&gt;2&lt;/sub&gt;Cl</td>
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<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td>224&lt;sup&gt;e&lt;/sup&gt;</td>
<td>C&lt;sub&gt;11&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;ClSO</td>
<td>33-35.5°C</td>
</tr>
<tr>
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<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td>228.0300&lt;sup&gt;d&lt;/sup&gt;</td>
<td>C&lt;sub&gt;11&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;D&lt;sub&gt;1&lt;/sub&gt;ClSO</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>228.0309&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td>C&lt;sub&gt;11&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;D&lt;sub&gt;1&lt;/sub&gt;ClSO</td>
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<td>30.9</td>
<td>25.8</td>
<td>26.0</td>
<td>5.4</td>
<td></td>
<td>196.9906&lt;sup&gt;d&lt;/sup&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;DCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>120-121°C</td>
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<td>196.9908&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>4.1</td>
<td>3.9</td>
<td>54.1</td>
<td>94.6</td>
<td></td>
<td>240.9409&lt;sup&gt;d&lt;/sup&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;DBrCl</td>
<td>128-129°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>240.9403&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;DBrCl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Calculated from the product of gas chromatogram peak heights and peak widths at half height.
b) Micellar addition reaction was heated to 60°C. PMR showed no 33.
c) 33 was stirred at 20-25°C in DMSO or THF without KOTBu present and worked-up. PMR showed only 33. Injection of 33 and 33 recovered from blank reactions results in thermal opening of the ring.
d) High resolution mass spectral data of products preparative gas chromatographed from d<sub>6</sub>-DMSO reaction.
e) Parent ion of low resolution mass spectrum of preparative gas chromatographed product of DMSO reaction.
f) Reactions were carried out at 20-25°C.
g) Calculated mass.
analysis of the halonaphthalenes showed no deuterium incorporation.

In tetrahydrofuran the reaction of 1-bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane 33 with potassium-t-butoxide produced three products: 19.2% 2-chloronaphthalene 45; 26.0% 2,3-dichloronaphthalene 57 and 54.1% 2-bromo-3-chloronaphthalene 58. These materials were isolated by thin layer chromatography, followed by preparative gas chromatography and identified by their mass spectra and melting points. No 2-chloronaphthalene 45 could be detected in the reaction of a 19:1 mixture of 2-bromo-3-chloronaphthalene 56 and 2,3-dichloronaphthalene 57 with potassium-t-butoxide in tetrahydrofuran under identical conditions.

When dimethyl sulfoxide was used as solvent the reaction of

\[
\begin{align*}
\text{33} & \xrightarrow{KOTBu, THF} \overset{45}{\text{Cl}} + \overset{57}{\text{Cl}} + \overset{56}{\text{Br}} + \text{Tar} \\
\text{33} & \xrightarrow{KOTBu, DMSO} \overset{45}{\text{Cl}} + \overset{57}{\text{Cl}} + \overset{56}{\text{Br}} + \overset{63}{\text{Cl}} + \text{Tar}
\end{align*}
\]
1-bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane \( \text{a} \) with potassium tert-butoxide became considerably more complex. Five products were isolated and identified: 59.2\% yield of 2-chloronaphthalene \( \text{b} \); 5.5\% yield of \( \text{C}_{11}\text{H}_{9}\text{ClSO} \text{c} \) (mp: 33-35\(^\circ\)C); 0.4\% yield of \( \text{C}_{11}\text{H}_{9}\text{ClSO} \text{d} \); 30.9\% yield of 2,3-dichloronaphthalene \( \text{e} \) (mp: 120-121\(^\circ\)C) and 4.1\% yield of 2-bromo-3-chloronaphthalene \( \text{f} \) (mp: 128-129\(^\circ\)C). When \( \text{d}_6 \)-dimethyl sulfoxide was used the majority of the 2-chloronaphthalene \( \text{g} \).

Table IV. Percent Deuteration of Products from Reaction 35

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \text{d}_0 )</th>
<th>( \text{d}_1 )</th>
<th>( \text{d}_2 )</th>
<th>( \text{d}_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{a} )</td>
<td>11.4</td>
<td>30.4</td>
<td>50.2</td>
<td>8.1</td>
</tr>
<tr>
<td>( \text{b} )</td>
<td>55.8</td>
<td>44.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>( \text{c} )</td>
<td>16.4</td>
<td>73.1</td>
<td>10.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

incorporated two deuterium atoms. Deuterium incorporation of byproduct \( \text{h} \) was not determined, however, byproduct \( \text{i} \) contained four deuterium atoms, whereas the majority of \( \text{j} \) and \( \text{k} \) each contained one deuterium atom.

The location of the deuterium atoms in 2-chloronaphthalene \( \text{a} \) was accomplished by assignment of peaks in the \( ^{13}\text{C} \) carbon magnetic resonance spectrum. The recent studies of monosubstituted naphthalenes by Kitching, Bulpitt, Doddrell and Adcock\(^5\) provided a reference for the effects observed on deuteration of naphthalenes. As expected, the deuterated material appears to contain two less peaks than the fully protonated 2-chloronaphthalene \( \text{a} \). Since deuterium has a spin
Table V. $^{13}$Carbon Assignments$^a$ of 2-Chloronaphthalene 45 and $d_2$-2-Chloronaphthalene from Reaction 35

<table>
<thead>
<tr>
<th>Carbon</th>
<th>2-Chloronaphthalene</th>
<th>1,3-Dideutero-2-chloronaphthalene</th>
<th>Δ Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126.35 ± 0.03$^b$</td>
<td>-</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>131.31$^c$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>126.50$^b$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>129.23</td>
<td>129.06</td>
<td>-0.17 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>127.54$^e$</td>
<td>127.51$^e$</td>
<td>-0.03</td>
</tr>
<tr>
<td>6</td>
<td>125.84</td>
<td>125.82</td>
<td>-0.02</td>
</tr>
<tr>
<td>7</td>
<td>126.64$^e$</td>
<td>126.64$^e$</td>
<td>0.00</td>
</tr>
<tr>
<td>8</td>
<td>126.81</td>
<td>126.74</td>
<td>-0.07</td>
</tr>
<tr>
<td>9</td>
<td>133.78</td>
<td>133.66</td>
<td>-0.12</td>
</tr>
<tr>
<td>10</td>
<td>131.41</td>
<td>131.39$^c$</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

a) Recorded on a Varian XL-100 spectrometer at 25.2 MHz. Chemical shifts are in ppm (error of ± 0.03 ppm) downfield from TMS; all spectra were taken in CDC1$_3$ and corrected using $\delta_{TMS} = \delta_{CDCl_3} + 76.90$ ppm.
b) Assignments could be reversed.
c) Approximately one-third the intensity of C9 and C10.
d) Obscured by the breadth of C10.
e) Assignments could be reversed.
quantum number I = 1, it couples with atoms (such as $^{13}$C) with spin quantum number I = 1/2 to form a 1:1:1 triplet. The intensity of each peak is therefore one-third the intensity of a non-deuterated peak. Furthermore, the loss of nuclear Overhauser effects broaden these three peaks, such that in a natural abundance spectrum it is often difficult to see a C-D triplet above the noise level.

The smaller linewidth of quaternary carbon signals allows the assignment of the C2, C9 and C10 peaks. In the non-quaternary sample, it will be noted that one of the three quaternary signals is less intense than the other two. This less intense peak at 131.31 ppm is assigned C2, the carbon bearing chlorine. In the deuterated sample the breadth of the adjacent peak obscures this C2 peak. Calculations from known chemical shifts of naphthalene, and shift effects observed on chlorination of benzenes indicate the lowest field signal of the spectrum to be the C9 peak. The peak at 133.78 ppm in the non-deuterated sample is therefore assigned this position. It will be noted that this C9 peak is shifted -0.12 ppm in the deuterated spectrum as expected of a carbon ortho to a carbon bearing deuterium. This establishes the location of one deuterium atom in the C1 position.

Of the non-quaternary signals, the highest field peak, according to calculation, is expected to arise from the 4-position. In the deuterated spectrum it also is shifted -0.17 ppm, indicating a carbon bearing deuterium adjacent to C4. Since the only non-quaternary carbon ortho to C4 is C3, the second deuterium atom must be located in the 3-position.

Carbon atoms meta to the carbon bearing deuterium are broadened by coupling of J(CCCD) approximately 0.04 ppm. The C10
signal, meta to two deuterium bearing carbons, has a linewidth of about 0.15 ppm. The C1 and C3 signals are not visible above the noise level due to both J(CD) and mutual J(CCCD) coupling. Finally, the signal at 126.81 ppm is shifted -0.07 ppm in the deuterated sample. This is a small, but significant shift, possibly arising from a peri effect between the deuterated C1 position and C8. C5, C6 and C7 cannot rigorously be assigned to the remaining peaks. However, the trend among 2-substituted naphthalenes indicates that generally the C6 position gives the highest field peak. Nonetheless, since the deuterium atoms cannot chemically be introduced under these reaction conditions into the second ring, we feel confident that the assignment of peaks is consistent with 1,3-deuterium labeling of the 2-chloronaphthalene 45.

As a check on the possible intermediacy of either 2-bromo-3-chloronaphthalene 56 or 2,3-dichloronaphthalene 57, a 19:1 mixture of these two compounds was subjected to identical reaction

Reaction 36

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl} \\
\text{Br} & \quad \text{Br} \\
\text{KO\textsubscript{t}Bu} & \quad \text{DMSO} \\
\text{Cl} & \quad \text{Tar}
\end{align*}
\]

conditions in d\textsubscript{6}-dimethyl sulfoxide. After thirty minutes reaction time all the dihalonaphthalenes were consumed leaving only 2-chloronaphthalene 45. Mass spectral analysis showed 1.5% nondeuterated, 40.2% monodeuterated, 38.9% dideuterated and 19.4% trideuterated 2-chloronaphthalene 45. This was compared with 11.4% nondeuterated 30.4% monodeuterated, 50.2% dideuterated and 8.1% trideuterated 2-chloronaphthalene 45 arising from the d\textsubscript{6}-dimethyl sulfoxide reaction of 1-bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane 32 with potassium
Table VII. $^{13}$Carbon Assignments$^a$ of 2-Chloronaphthalene$^b$ and Deuterated-2-chloronaphthalene from Reaction 36

<table>
<thead>
<tr>
<th>Carbon</th>
<th>2-Chloronaphthalene</th>
<th>Deuterated-2-chloronaphthalene</th>
<th>$\Delta$ Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126.35 $\pm$ 0.03$^b$</td>
<td>126.35 $\pm$ 0.03$^b$</td>
<td>0.00 $\pm$ 0.04</td>
</tr>
<tr>
<td>2</td>
<td>131.31$^c$</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>126.50$^b$</td>
<td>126.40$^b$</td>
<td>-0.10</td>
</tr>
<tr>
<td>4</td>
<td>129.23</td>
<td>129.09</td>
<td>-0.14</td>
</tr>
<tr>
<td>5</td>
<td>127.54$^e$</td>
<td>127.51$^e$</td>
<td>-0.03</td>
</tr>
<tr>
<td>6</td>
<td>125.84</td>
<td>125.84</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>126.64$^e$</td>
<td>126.62$^e$</td>
<td>-0.02</td>
</tr>
<tr>
<td>8</td>
<td>126.81</td>
<td>126.76</td>
<td>-0.05</td>
</tr>
<tr>
<td>9</td>
<td>133.78</td>
<td>133.69</td>
<td>-0.09</td>
</tr>
<tr>
<td>10</td>
<td>131.41</td>
<td>131.31$^c$</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

a) Recorded on a Varian XL-100 spectrometer at 25.2 MHz. Chemical shifts are in ppm (error of $\pm$ 0.03 ppm) downfield from TMS; all spectra were taken in CDC13 and corrected using 

$\delta_{\text{TMS}} = \delta_{\text{CDC13}} + 76.90$ ppm.

b) Assignments could be reversed.
c) Approximately one-third the intensity of C9 and C10.
d) Obscured by the breadth of C10.
e) Assignments could be reversed.
Table VI. Percent Deuteration of 2-Chloronaphthalene \(45\) by Exchange and from Reactions 35 and 36

<table>
<thead>
<tr>
<th>Reaction</th>
<th>(d_0)</th>
<th>(d_1)</th>
<th>(d_2)</th>
<th>(d_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exchange</td>
<td>59.2</td>
<td>30.5</td>
<td>10.3</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td>11.4</td>
<td>30.4</td>
<td>50.2</td>
<td>8.1</td>
</tr>
<tr>
<td>36</td>
<td>1.5</td>
<td>40.2</td>
<td>38.9</td>
<td>19.4</td>
</tr>
</tbody>
</table>

\(\text{t-Butoxide.} \quad ^{13}\text{Carbon magnetic resonance showed the majority of deuteration from the dihalonaphthalene reaction in the one and three positions with some in the four position (a shift of -0.10 ppm in the C10 signal). To further complicate this reaction 2-chloronaphthalene \(45\) itself was found to exchange protons with solvent under these reaction conditions. The recovered 2-chloronaphthalene \(45\) contained 59.2% nondeuterated, 30.5% monodeuterated and 10.3% dideuterated material. These percentages of deuterated materials were calculated from the intensities of the m/e 160 through 169 mass spectral peaks after accounting for \(^{13}\text{C}\) and chlorine isotopes. The fragmentation of 2-chloronaphthalene \(45\) in this mass range was also taken into account.} \n
1-Bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane \(33\) was also reacted with lithium diethylamide in ether at -15°C. This

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} & \quad \text{LiN(C_2H_5)_2} & \quad \text{Cl} \\
\text{Br} & \quad \text{Ether} & \quad -15^\circ C & \quad + S_4 + S_2 & \\
\end{align*}
\]

\(33\) \quad \(45\)
reaction was also quite complex, producing 2-chloronaphthalene \( \text{C}_{11}\text{H}_{8}\text{Cl} \) in 17.3% yield, and four diethylamine containing compounds in yields of 3.7% (base peak m/e 184) \( \text{C}_{22}\text{H}_{25}\text{Cl} \), 19.5% (base peak m/e 218) \( \text{C}_{24}\text{H}_{27}\text{Cl} \), 34.6% (base peak m/e 218) \( \text{C}_{24}\text{H}_{27}\text{Cl} \), and 24.8% (base peak m/e 218) \( \text{C}_{24}\text{H}_{27}\text{Cl} \).

All the reactions of the bicyclo[3.1.0]hexane systems as well as the bromobenzene reaction and the dihalonaphthalene reactions reported in this thesis produced a considerable (30-50% yield) amount of tarry material which was not analyzed.
DISCUSSION

During the initial stages of this investigation, considerable effort was devoted to the development of efficient syntheses of the target halogenated bicyclo[3.1.0]hexanes.

Although the synthesis of 3,5-dibromocyclopentene \[ \text{27} \] is straightforward, the lithium aluminum hydride reduction is apparently quite complex. The explosions and fires observed by Johnson and Keiser \[ \text{39} \] occurred one hour after the addition of \[ \text{27} \] was complete and during the time the reaction was warming to room temperature. This sounds as if the reaction either proceeds with an induction period or begins (to an appreciable extent) only at temperatures above 0°C. If an induction period were the cause one could minimize the explosion hazard by the addition of a small amount of 3,5-dibromocyclopentene \[ \text{27} \] followed by the rest of the material at a carefully controlled rate, only after the induction period. On the other hand, if a critical temperature is the problem, then the slow addition of 3,5-dibromocyclopentene \[ \text{27} \] at room temperature should solve the explosion problem. Since in our hands, this reaction at room temperature did not
explode, and since both Johnson and Keiser\textsuperscript{39} and Bartlett and Rice\textsuperscript{38} did not always have an explosion, the hazard appears to be a function of a critical temperature.

The elimination of hydrogen bromide from the 1,2-dibromo-indan\textsuperscript{51} presents two basic problems. First, since 83\% of the dibromide

\begin{equation}
\text{Reaction 39}
\end{equation}

is in the trans configuration, the hydrogen bromide elimination is formally a cis elimination. Secondly, three possible bromoindene isomers

\begin{equation}
\text{Exist 52, 68 and 69.}
\end{equation}

The use of lithium chloride or lithium bromide-lithium carbonate has been quite successfully employed by Corey and Hortman\textsuperscript{46} in the synthesis of \(\alpha,\beta\)-unsaturated ketones, however it was not previously applied to the synthesis of vinylbromides. Holysz\textsuperscript{51} studied the lithium chloride reaction and proposed two mechanisms. In the concerted, four center reaction, the lithium chloride first forms a complex with the solvent dimethylformamide. Besides the olefin product a negatively charged \(\text{LiX}_2^-\) ion and an ammonium ion are formed. This mechanism would not account for the exchange of chlorine for
Reaction 40

bromine in the lithium bromide attempt on the dibromoindan 51. His second mechanism is a stepwise process, beginning with an $S_N^2$ attack of halide on the alkyl bromide, followed by trans elimination. This

Reaction 41

accounts both for the halide exchange observed, and for the success of
the reaction. This reagent was also successfully applied in the synthesis of 1,2-dihydro-3-bromonaphthalene \( \text{55} \).

Compared with the standard carbene addition, the micellar method is far superior in applicability, yield and cost. The base, sodium hydroxide, is transferred from the aqueous phase to the chloroform by the micellar agent - a high molecular weight trialkyl ammonium salt. The only limitation is the formation of bis adducts

\[ \text{Reaction 42} \]

![Reaction diagram]

when two double bonds are present. Besides demonstrating through competitive experiments, that micellar agents actually form free carbene and not carbenoids, Skell and Chodorow \( \text{52} \) showed that micellar generated dichlorocarbene and chlorobromocarbene could be efficiently added to slightly deactivated double bonds - stilbenes and acetylenes.

In a paper discussing the effect of substitution on standard carbene additions, Parham and Wright \( \text{47} \) concluded that the more electron withdrawing the substituent on the double bond, the less reactive it is to dichlorocarbene addition. Without demonstrating the isomeric purity of bromoindene, they isolated a 1.6% yield of a bromochloronaphthalene. In light of their discussion of substituent effects, any 3-bromoindene \( \text{62} \) would have preferentially added the dichlorocarbene to give 1-bromo-3-chloronaphthalene \( \text{70} \) on heating. Apparently the first successful synthesis and characterization of a dichlorocarbene adduct of a deactivated double bond is the 1-bromo-3,4-benzo-6,6-dichloro-
bicyclo[3.1.0]hexane 22. So far, in this laboratory, Blakeney and Reed have been unsuccessful in applying this micellar method to 1,2-dihydro-3-bromonaphthalene 55 and 9-bromophenanthrene 58 respectively.

Besides the spectroscopic data, a sodium-ammonia reduction of the halogens was used to demonstrate the carbon skeleton of 32 and 22. Both, however, produced significant yields of byproducts -

Reactions 43 and 44

tetralin 44 and 1,4-dihyronaphthalene 61 respectively. The 32 used in the sodium-ammonia reduction was shown by $^{13}$C carbon spectroscopy to be an impure sample, and the byproduct probably arose from an impurity. (After recrystallization a clean $^{13}$C carbon spectrum of 32 was obtained.) The 1,4-dihyronaphthalene 61 was however, a real product from pure 33. It was probably not due to rearrangement of
on the gas chromatography column, since reinjection of collected showed no 1,4-dihyronaphthalene. Although this type of rearrangement has not observed before, this starting material is the first example that could form a relatively stable benzyl and allyl radical.

\[ \text{Reaction 45} \]

\[ \text{3} \xrightarrow{\text{Cl, Br}} \text{4} \xrightarrow{\text{Cl}} \text{5} \xrightarrow{\text{Cl}} \text{6} \]

Myers, Stroebel, Ortiz de Montellano and Gardner propose that this reduction is initiated by the addition of an electron to form the radical-anion. The sodium-ammonia reduction of 3 could therefore be visualized as proceeding through the radical-anion 7.

Although 3 is quite stable in the non-polar solvents, tetrahydrofuran, ether, carbon tetrachloride and chloroform, in the highly polar dimethyl sulfoxide a solvolysis producing 2,3-dichloro- and 2-bromo-3-chloronaphthalene was followed with PMR spectroscopy. The indene adduct was however, stable even in dimethyl sulfoxide.

From a graphical analysis of the peak heights appears to solvolyze in a unimolecular fashion, producing peaks only in the aromatic region. Exact values of chemical shifts cannot be given since an internal standard of tetramethylsilane was omitted in order to not obscure the cyclopropyl region of the spectrum. After fourteen hours the spectrum
showed a slight variation from that recorded at 165 minutes. It was
at fourteen hours that a gas chromatographic analysis of the products
was obtained. A mechanism consistent with these data includes loss
of endo-chloride followed by the sequence of steps illustrated below.

\[ \text{Reaction 46} \]

The results obtained from reactions of 33 in dimethyl sulfoxide with
potassium t-butoxide are of course colored by this solvolysis. However,
the half-life at 40°C measured from the PMR data is forty-eight minutes,
whereas solutions of 33 in dimethyl sulfoxide were made-up within five
minutes of the time of addition to the reaction and the addition
period was not more than ten minutes.

In the hope of obtaining evidence for the existence of the
parent \textit{m}-benzyne 2, 33 was subjected to potassium t-butoxide in
dimethyl sulfoxide or tetrahydrofuran. It was hoped that the following eliminations and isomerizations would occur:

Reaction 47

However no benzene \( \text{C}_6 \text{H}_6 \) was detected. The primary product followed the Woodward-Hoffmann allowed disrotatory cyclopropyl ring opening.

Reaction 48

It was hoped that stabilization of one of the double bonds with an aromatic ring, might favor the formation of a \( \text{m} \)-benzyne \( \text{C}_6 \text{H}_5 \). For this reason, \( \text{C}_6 \text{H}_5 \text{Cl} \) was synthesized and treated with potassium \( \text{t} \)-butoxide in dimethyl sulfoxide or tetrahydrofuran. Again, the formation of a hydrocarbon, naphthalene \( \text{C}_{10} \text{H}_{8} \) was to be taken as evidence for the 1,3-aryne intermediate. However, its formation was probably not favored since the aromatic ring had to be disturbed. Nevertheless, 0.1% yield of naphthalene \( \text{C}_{10} \text{H}_{8} \) was formed. Once again the primary product, 2-chloronaphthalene \( \text{C}_{10} \text{H}_{7} \text{Cl} \), was due to the Woodward-Hoffmann allowed ring opening.
The other products, naphthol 47 and methoxynaphthalene 48, are probably decomposition products of t-butyl naphthyl ether 72, possibly formed by the action of t-butoxide on 2-chloronaphthalene 45. No naphthalene 46 was formed when 2-chloronaphthalene 45 was subjected to identical conditions of base concentration in dimethyl sulfoxide.

An interesting sidelight of this reaction was the gradual formation of a blue band on the silica gel thin layer chromatography plate, in the purification of the products of the reaction of 32. Parham and Reiff 42 report the formation of a chloroazulene 49 in the reaction of indenyl sodium 50 with chloroform, which they believed arose from
the dichlorocarbene addition to the aromatic ring. If this blue band is the same as Parham and Reiff's blue material, then it surely cannot arise as they suggest. The indene adduct 32 was pure, at least by PMR and $^{13}$C spectroscopy and melting point. Perhaps, as Chamberlain (this laboratory) suggested, a carbene which inserts into the aromatic ring is formed when 32 is treated with base. This would result in a bicyclobutane 73 intermediate. The thermally allowed ring openings would lead to 2-chloroazulene 74 and 1-chloronaphthalene 75. The implication from the gradual formation of the blue band is that the bicyclobutane 73 intermediate opens slowly at room temperature. The conversion of azulenes to naphthalenes was well established already.
in 1947 by Heilbronner, Plattner and Wieland.\textsuperscript{54} The mechanism of conversion is only currently being investigated by Scott,\textsuperscript{55} who also proposes a bicyclobutane intermediate.

From the results so far, it becomes obvious that the primary mode of reaction of 6,6-dichlorobicyclo[3.1.0]hexanes is the thermally allowed ring opening. The chances of obtaining evidence for a \textit{m}-benzyne would therefore be greatly improved if a more favorable alternative were present. For this reason, a halogen was introduced in the bridgehead position, to favor elimination over ring opening.

As expected, the yield of 2-chloronaphthalene \textsuperscript{45} most likely arising from the \textit{m}-naphthyne \textsuperscript{34} was increased to 17.9% when \textsuperscript{33} was reacted with potassium tert-butoxide in tetrahydrofuran. The starting material \textsuperscript{33} was stable in tetrahydrofuran, so the byproducts 2,3-dichloronaphthalene \textsuperscript{57} and 2-bromo-3-chloronaphthalene \textsuperscript{58} were produced as a result of base induced ring opening reactions. In order to rule out the possible intermediacy of the byproducts in the formation of 2-chloronaphthalene \textsuperscript{45}, a mixture of 2,3-dichloronaphthalene \textsuperscript{57} and 2-bromo-3-chloronaphthalene \textsuperscript{58} was subjected to identical reaction conditions (potassium tert-butoxide in tetrahydrofuran). The dihalo-
naphthalenes were recovered in quantitative yield in their original proportions. The 2-chloronaphthalene $^{45}$ is therefore an independent product of the reaction of $^{33}$ with potassium t-butoxide in tetrahydrofuran.

The intermediate m-naphthylene $^{34}$ appears to react as a diradical $^{35}$ by abstracting hydrogen atoms from solvent to form 2-chloronaphthalene $^{45}$. More convincing evidence would demonstrate that the one and three positions are indeed the radical centers. Since perdeuterated tetrahydrofuran is prohibitively expensive in the quantities needed the reaction was attempted first in nondeuterated and then in perdeuterated dimethyl sulfoxide. 2-Chloronaphthalene $^{45}$ was produced in 33.3% yield. As might be expected, because the base is more soluble in dimethyl sulfoxide than in tetrahydrofuran, the yield is higher. Once again 2,3-dichloronaphthalene $^{57}$ and 2-bromo-3-chloronaphthalene $^{56}$ are produced as byproducts, as well as two isomers of $C_{11}H_{9}ClSO$ $^{62}$ and $^{63}$. The two dimethyl sulfoxide incorporating isomers might arise, either from recombination of radicals formed when the m-naphthylene $^{35}$ abstracts a hydrogen atom from solvent, or possibly from the o-naphthylene $^{76}$ arising from 2-bromo-3-chloronaphthalene $^{56}$ as described by Bradshaw and Hales. $^{30}$ When the deuterated solvent was used the majority of the 2-chloronaphthalene $^{45}$ contained two deuterium atoms. The $^{13}$ carbon magnetic resonance spectrum demonstrated that the deuterated positions were indeed the two adjacent to the carbon bearing chlorine.

Once again the intermediacy of the dihalonaphthalene byproducts was checked. In this case, however, the identical reaction conditions totally consumed the dihalonaphthalenes producing only 2-chloro-
naphthalene. The reaction was repeated in deuterated dimethyl sulfoxide to determine the extent and positions of deuteration. \(^{13}\text{CMR}\) demonstrated labeling in the one and three positions, as well as considerable labeling of the four position. It was also determined that 2-chloronaphthalene itself exchanges protons with solvent to some extent.

Nevertheless, it can be demonstrated that not all of the 2-chloronaphthalene can be accounted for through the intermediacy of the dihalonaphthalenes. Since no trideuterated material is observed in the exchange of 2-chloronaphthalene, the trideuterated material from 2-bromo-3-chloronaphthalene and 2,3-dichloronaphthalene must arise by exchange in the one position prior to reduction of the bromine. For this reason, the 8.1% trideuterated material in the reaction of and gives an upper limit to the amount of 2-chloro-
Table VIII. Corrections to the Percent Deuteration of 2-Chloronaphthalene from Reaction 35

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$d_0$</th>
<th>$d_1$</th>
<th>$d_2$</th>
<th>$d_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exchange</td>
<td>59.2</td>
<td>30.5</td>
<td>10.3</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td>11.4</td>
<td>30.5</td>
<td>50.2</td>
<td>8.1</td>
</tr>
<tr>
<td>36</td>
<td>1.5</td>
<td>40.2</td>
<td>38.9</td>
<td>19.4</td>
</tr>
<tr>
<td>a</td>
<td>0.6</td>
<td>16.9</td>
<td>16.3</td>
<td>8.1</td>
</tr>
<tr>
<td>35 - a</td>
<td>10.8</td>
<td>13.5</td>
<td>33.9</td>
<td>0.0</td>
</tr>
<tr>
<td>b</td>
<td>0.2</td>
<td>5.1</td>
<td>8.7</td>
<td>8.1</td>
</tr>
<tr>
<td>35 - b</td>
<td>11.2</td>
<td>25.3</td>
<td>41.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

a) Correction for 2-chloronaphthalene arising from reduction of dihalonaphthalenes 56 and 57, assuming all the trideuterated material arises from reduction.

b) Correction a, also taking into account that 55.8% of the 2-bromo-3-chloronaphthalene 56 is nondeuterated and 44.2% is monodeuterated before reduction.

naphthalene 45 arising from reduction of bromine or chlorine. The Reaction 55
only other way trideuterated material might arise is by exchange in 
$^{22}$ prior to elimination. Subtracting the upper limit percentages, 
due to reduction of the dihalonaphthalenes leaves: 10.8% nondeuterated, 
13.5% monodeuterated and 33.9% dideuterated - an appreciable amount 
of material dideuterated in the one and three positions not accounted 
for by reduction of dihalonaphthalenes. If one takes into account 
that 44.2% of the 2-bromo-3-chloronaphthalene $^{26}$ produced in the 
reaction of $^{22}$, already has one deuterium atom before being reduced, 
then the percentage of 1,3-dideuterated-2-chloronaphthalene $^{45}$ 
unaccounted for increases. This material most likely arises from 
$m$-naphthylene $^{25}$.

Another observation is that the amount of nondeuterated 
material from $^{22}$ is ten times the amount from the dihalonaphthalenes. 
If one now applies the deuterium distribution figures from 2-chloro-
naphthalene $^{45}$ itself to the figures from $^{22}$, one obtains an upper 
limit for the amount of nondeuterated material present immediately 
after the reaction of $m$-naphthylene $^{25}$: 18.2%. That is, if one 
assumes the formation of $m$-naphthylene $^{25}$ is instantaneous, nearly 20% 
of the material is nondeuterated immediately after the reaction of 
$m$-naphthylene $^{25}$. This nondeuterated material must arise without 
exchange of protons with solvent. Two possible mechanisms exist

Reaction 56
- either a chlorine-bromine elimination, possibly arising from dimethyl abstraction of a bromonium ion followed by loss of a chloride ion. Rearrangement of the intermediate \( \text{32} \) could yield unlabeled 2-chloronaphthalene \( \text{45} \). Reaction of the \( m \)-naphthylene \( \text{35} \) with \( t \)-butanol in the solvent pool would also result in unlabeled 2-chloronaphthalene \( \text{45} \).

\[ \text{Reaction 57} \]

\[ \begin{array}{ccc}
\text{Cl} & \text{Cl} & \text{Br} \\
\text{2KO} & \text{Bu} & \text{d6-DMSO} \\
\end{array} \rightarrow \text{Cl} \]

The reaction of \( \text{32} \) with lithium diethylamide in ether at \(-15^\circ\text{C}\) appears to be equally complex. The only identified product was a 17.3\% yield of 2-chloronaphthalene \( \text{45} \). Four other products were isolated by gas chromatography and identified as diethylamides by their mass spectra.

Ideally, the total suppression of the ring opening reaction should eliminate the formation of the dihalo byproducts. That is, the ideal molecule for study would be \( \text{80} \) or \( \text{81} \) or \( \text{82} \) for the parent.
m-benzyne \( \mathcal{Z} \). So far, no reaction is known which will either add mono-
chlorocarbene to a deactivated double bond or which will selectively 
reduce off the \textit{endo} chlorine.

From the results obtained in this study of bicyclo[3.1.0] 
hexanes with base, it would appear that a 1,3-dehydroaromatic 
compound is formed. Furthermore, it apparently reacts as a diradical, 
producing a reduced compound. In view of the large number of partially 
explained reductions, none of which began with materials lacking \textit{meta} 
hydrogens, perhaps the formation of m-benzyne \( \mathcal{Z} \) ought to be given more 
consideration as a competitive side reaction of o-benzyne \( \mathcal{Z} \) formation. 
With this in mind, bromobenzene \( \mathcal{Z} \) was reacted with sodium amide in 
liquid ammonia. A 16% yield of aniline \( \mathcal{Z} \) and a 2% yield of benzene \( \mathcal{Z} \) 
was produced. It is quite possible that reduced products in other 
o-benzyne \( \mathcal{Z} \) producing reactions have also been overlooked. It would 
be quite interesting to reinvestigate o-benzyne \( \mathcal{Z} \) reactions of 
compounds both containing and lacking \textit{meta} hydrogens, for traces of 
reduced materials.

Probably m-pyridynes ought also to be given more serious 
thought. In heteroaromatic chemistry it is well accepted that the 
4-position is most acidic, yet the formation of 2,4-pyridyne, for 
example, is not discussed. Since radical intermediates are known to 
produce rearranged products and since m-naphthyne \( \mathcal{S} \) appears to behave 
as a diradical, perhaps m-pyridynes also opening to diradicals are 
involved in the \textit{meta} rearrangement and other skeletal rearrangements 
observed by van der Lans, den Hertog and van Veldhuizen.\textsuperscript{19}
Proton magnetic resonance spectra were recorded as carbon tetrachloride or deuterochloroform solutions on a Varian A 56/60 spectrometer. Chemical shifts are reported in parts per million relative to an internal standard of tetramethyilsilane. A Varian XL-100 spectrometer was used to obtain the $^{13}$C carbon magnetic resonance spectra. Infrared spectra were recorded either as neat liquids or as potassium bromide pellets on a Beckman IR-8 instrument. A Consolidated Electrodynamics Corporation 21-1100 high resolution mass spectrometer provided the mass spectral data. Cyclohexane solutions were used to record the ultraviolet spectra on a Cary Model 17 spectrometer. Gas chromatographs were all equipped with thermal conductivity detectors and peak areas were determined from the product of the peak height by the width at one-half the height. It was assumed that area ratios were proportional to mole ratios.

Most of the preparative gas chromatography was done on a Varian Aerograph A-700, whereas the analytical gas chromatography was done on either the Hewlett-Packard 700 or 5700 instrument. Melting points and boiling points are uncorrected.

Potassium $t$-butoxide and $90\%$ n-butyll lithium in hexane were obtained from PCR Incorporated and used without further purification. Ether and tetrahydrofuran were freshly distilled from sodium-benzophenone ketal, and dimethyl sulfoxide was freshly distilled
from calcium hydride. d₆-Dimethyl sulfoxide was obtained in 99.5% purity from Stohler Isotope Chemicals. Unless otherwise indicated, all reactions were carried out in a nitrogen atmosphere.

**Preparation of 3,5-Dibromocyclopentene**: 3,5-Dibromocyclopentene was prepared according to the procedure of Bartlett and Rice. A solution of cyclopentadiene (175 gm; 2.66 moles) in 125 ml of pentane was placed in a 1-liter three-necked flask equipped with a stirrer, addition funnel and a drying tube and cooled to -30°C. While keeping the temperature of the reaction mixture below -30°C, 427 gm (2.66 moles) of bromine dissolved in 225 ml of pentane was added slowly over a two hour period. The reaction mixture was poured into a 1-liter Erlenmeyer flask, cooled to -80°C and the solvent decanted. Enough ether was added to dissolve the residue. The 3,5-dibromocyclopentene was immediately subjected to the lithium aluminum hydride reduction.

**Preparation of 4-Bromocyclopentene**: Although the procedure of Bartlett and Rice was followed uneventfully in our laboratory, Johnson and Keiser extend a note of caution in the use of this procedure.

The ether solution of 3,5-dibromocyclopentene from the previous procedure was added over a two hour period (via an addition funnel fitted on a 2-liter three-necked flask equipped with a stirrer and a reflux condenser) to 44 gm (1.3 moles) of lithium aluminum hydride in 570 ml of ether. External cooling to maintain a reaction temperature around 25°C, from an ice bath was maintained throughout
the addition. The reaction mixture was stirred overnight and finally was refluxed 36 hours. It was filtered through glass wool and the filtrate poured over ice. The layers were separated, the ethereal layer dried over calcium chloride, and the solvent removed in vacuo. The residue was vacuum distilled and collected at 43°C (35 mm/Hg), giving a 10% yield of the desired 4-bromocyclopentene 38.

**Preparation of 3-Bromo-6,6-dichlorobicyclo[3.1.0]hexane 31:** A micellar carbene addition was used to obtain 31 from 4-bromocyclopentene 38. In a 500-ml three-necked flask, fitted with a mechanical stirrer, reflux condenser and addition funnel, 35 ml (0.44 mole) of alcohol free chloroform, 18.23 gm (0.124 mole) of 4-bromocyclopentene 38 and 0.33 gm (0.9 mmole) of hexadecyltrimethylammonium bromide were stirred together. A solution of 46 gm (2 moles) of sodium hydroxide in 77 ml of water was added dropwise. After stirring overnight at room temperature, the reaction mixture was poured over ice and acidified with 10% sulfuric acid. The product was extracted into ether, washed with water and saturated sodium chloride solution and dried with sodium sulfate. After removal of the ether, vacuum distillation afforded 2.03 gm (8.8 mmole) of distillate at 47°C (0.6 mm/Hg).

A standard potassium t-butoxide, chloroform, substrate (molar ratio 2.5:3:1) in pentane at 0°C carbene addition also gave only a 7% yield of 31.

**Preparation of 1,2-Dibromoindan 51:** 1,2-Dibromoindan 51 was prepared according to the procedure of Winsten and Roberts. A 500-ml three-
necked flask, equipped with a mechanical stirrer, an alcohol thermometer and an addition funnel was purged with nitrogen. Freshly distilled indene \( \frac{43}{43} \) (34.8 gm; 0.3 mole) in 150 ml of dry ether was placed in the flask and cooled with an ice-salt bath to -5\(^\circ\)C. While maintaining the reaction temperature below 0\(^\circ\)C, 17 ml (approximately 0.3 mole) of bromine was added dropwise until the orange color persisted. After placing the reaction solution in a separatory funnel, it was washed twice with a saturated solution of sodium thiosulfate, twice with water and finally with saturated sodium chloride solution. The ethereal layer was dried over magnesium sulfate, filtered and the solvent removed. Collection of the distillate from 120-130\(^\circ\)C (4.5 mm/Hg) afforded 67.2 gm (81\% yield) of a cis-trans mixture of 1,2-dibromoindan \( \frac{51}{51} \).

**Preparation of 2-Bromoindene 52:** With reference to a procedure by Corey and Hortmann\(^ {46} \) hydrogen bromide was eliminated from 1,2-dibromoindan \( \frac{51}{51} \) using a suspension of lithium bromide and lithium carbonate in dimethylformamide. Commercial lithium bromide was heated under vacuum overnight to remove water and the dimethylformamide was stored over 4\(^\circ\)A molecular sieves. The suspension of 30 gm (0.34 mole) of lithium bromide and 40 gm (0.54 mole) of lithium carbonate in 500 ml of dimethylformamide was mechanically stirred under nitrogen in a 1-liter three-necked flask fitted with a condenser and an addition funnel. After bringing the dimethylformamide to reflux, 55.2 gm (0.2 mole) of 1,2-dibromoindan \( \frac{51}{51} \) was added dropwise over approximately thirty minutes. The reaction was refluxed an additional four hours and finally cooled to room temperature. After acidifying
the reaction mixture with 10% acetic acid, the product was extracted into ether. The ethereal layer was washed with water and the combined aqueous layers back-extracted with ether. Finally, the combined ethereal layers were washed several times with water and once with saturated sodium chloride solution. The solvent was removed in vacuo after drying the ethereal layer with anhydrous sodium sulfate. Distillation of the black tar at 77°C (2 mm/Hg) afforded 22.6 gm (58% yield) of a white solid. After recrystallization from methanol only one peak could be detected in the gas chromatogram using a 6 ft x \( \frac{1}{4} \) in. column of either 15% Carbowax 20M on Chromasorb P or 20% SE-30 on Chromasorb P.

Preparation of 2,3-Benzo-6,6-dichlorobicyclo[3.1.0]hexane: Although Parham, Reiff and Swartzentruber first prepared compound by adding dichlorocarbene (generated from chloroform in pentane using potassium tert-butoxide as the base) to indene, the overall yield based on indene employed in one cycle of the reaction is only 8.03%. Modification of the micellar carbene additions of Joshi, Singh and Pande resulted in an overall yield of 57%. In a 500 ml three-necked flask fitted with a reflux condenser, an efficient mechanical stirrer and an addition funnel, 0.528 gm (1.4 mmole) hexadecyltrimethylammonium bromide, 23.2 gm (0.2 mole) freshly distilled indene and 50 ml (0.63 mole) of alcohol free chloroform were stirred together under nitrogen. With external ice-bath cooling, 61.36 gm (1.53 mole) sodium hydroxide dissolved in 122 ml of water, was added dropwise over approximately thirty minutes. The ice-bath was allowed to warm to room temperature and the reaction was stirred overnight. After
acidifying the reaction mixture with 10% sulfuric acid, the product was extracted into ether and washed repeatedly with water. The ethereal layer was dried with magnesium sulfate; the drying agent removed by filtration and the solvent removed in vacuo, making sure that the product was never warmed above 50°C. The product was taken up in petroleum ether and filtered through a pad of alumina. Low temperature recrystallization from petroleum ether afforded 13.2 gm (57% non-optimized yield) of 32.

Preparation of 1-Bromo-3,4-benzo-6,6-dichlorobicyclo[3.1.0]hexane 33:
This compound was prepared in 27.2% yield from 9.75 gm (0.05 mole) of 2-bromoindene 52 using the procedure described above for compound 32. Reaction time at room temperature was increased to thirty-six to forty-eight hours and care was taken that neither the reaction mixture nor the product was ever warmed above room temperature. The product appears to be stable indefinitely in the freezer, however slowly opens to a mixture of 2,3-dichloro- 57 and 2-bromo-3-chloronaphthalenes 56 at room temperature.

When the reaction is carried out at 55 to 60°C for two hours a 1:19 mixture of 2,3-dichloro- 57 and 2-bromo-3-chloronaphthalenes 56 is obtained.

Preparation of 2-Bromo-3-chloronaphthalene 56: Compound 33 was heated in refluxing carbon tetrachloride twelve hours until nuclear magnetic resonance showed only aromatic protons. Removal of solvent, followed by recrystallization from ethanol-water yielded an inseparable (other than by preparative gas chromatography) mixture (1:19) of 2,3-dichloro-
and 2-bromo-3-chloronaphthalenes 56.

**Preparation of 2-Chloronaphthalene 45:** In a similar way, compound 32 was heated until only aromatic signals were detectable in the nuclear magnetic resonance spectrum. Gas chromatographic traces after recrystallization showed only 2-chloronaphthalene 45.

**Sodium-ammonia reduction of 32:** Sodium (1.82 gm; 79 mmole) was rapidly added in small pieces to approximately 100 ml of dry liquid ammonia in a 250 ml three-necked flask outfitted with a dry ice-acetone condenser, an Herschberg stirrer and an addition funnel. A solution of 32 (1.194 gm; 6 mmole) in 25 ml of dry ether was added dropwise over twenty minutes. The solution was stirred four hours and then treated with ammonium chloride until the blue color was discharged. After evaporation of the ammonia, water was added and the products extracted into ether. The ether was removed in vacuo and the two products separated by preparative gas chromatography on a 6 ft x 1/4 in. column of 20% SE-30 on Chromasorb P at 125°C. The reaction was quantitative producing 0.4874 gm (45.7% yield) of 2,3-benzobicyclo[3.1.0]hexane 43 and 0.5886 gm (54.3% yield) of tetralin 44.

**Sodium-ammonia reduction of 33:** After appropriately adjusting the quantities for the reduction of a third halogen atom (2.726 gm; 118.5 mmole sodium) 1.656 gm (6 mmole) of 33 was reacted as described above. The yields of two products were also quantitative: 0.2698 gm (16.6% yield) 2,3-benzobicyclo[3.1.0]hexane 43 and 1.355 gm (83.4% yield) of 1,4-dihydronaphthalene 61.
Reaction of 31 with potassium t-butoxide: In a carefully dried 50 ml three-necked flask equipped with an addition funnel, a mechanical stirrer and a reflux condenser, was placed 0.97 gm (8.7 mmole) of potassium t-butoxide and 15 ml of dimethyl sulfoxide. 3-Bromo-6,6-dichlorobicyclo[3.1.0]hexane 31 (0.8061 gm; 3.52 mmole) was added dropwise within ten minutes while maintaining the temperature below 20°C. After stirring at room temperature an additional one-half hour, the reaction was quenched with 20 ml of water. The products were extracted into 5 ml of pentane and dried over sodium sulfate. Gas chromatography of the pentane solution of the products on a 6 ft x \(\frac{1}{4}\) in. column of 20% dioctyl phthalate on Chromasorb P at a flow rate of 33 cc/min and temperature of 60°C showed three products, the major one (98.4%) of which had a retention time of 24 minutes and was identified as chlorobenzene 32. The two minor byproducts 42 and 41, 3 minute retention time (0.6% yield) and 6 minute retention time (1.0% yield), did not have the retention time of benzene (3.75 minutes) as shown in a simultaneous injection.

Reaction of 32 with potassium t-butoxide: After carefully drying a 50 ml three-necked flask fitted with a condenser, a mechanical stirrer and an addition funnel, 2.24 gm (20 mmole) of potassium t-butoxide was stirred under nitrogen in 15 ml of solvent. A solution of 32 (0.995 gm; 5 mmole) in 5 to 10 ml of solvent was added dropwise within ten minutes while maintaining the temperature between 15°C and 20°C. The reaction was allowed to continue stirring thirty minutes and then poured into ice-water. The products were extracted into ether and repeatedly washed with water. After drying the ethereal layer with
sodium sulfate, and removing the solvent in vacuo, the products were initially separated by thin layer chromatography on silica gel, using cyclohexane as developing solvent, followed by preparative gas chromatography on a 6 ft x ½ in. column of either 15% Carbowax 20M on Chromasorb P or 20% SE-30 on Chromasorb P. The products were identified by mass spectral data and melting points.

The reaction in dimethyl sulfoxide produced four products:
0.1% naphthalene 46; 99.2% 2-chloronaphthalene 45; and two byproducts, parent ion 158, mp 102-107°C in 0.2% yield and parent ion 144, mp 68-70°C in 0.4% yield.

The reaction in tetrahydrofuran produced only two products:
0.1% naphthalene 46 and 99.9% 2-chloronaphthalene 45.

When 32 was stirred in solvent either dimethyl sulfoxide or tetrahydrofuran, without potassium t-butoxide present and worked-up as described above, nuclear magnetic resonance showed only starting material.

**Reaction of 33 with potassium t-butoxide:** In a similar way, 33 was reacted with potassium t-butoxide in several solvents and the products analyzed as described above. Blank reactions (without base present) in tetrahydrofuran or diethyl ether showed only starting material 33 in the PMR spectrum. In dimethyl sulfoxide the starting material 33 solvolyzes to 1:19 2,3-dichloronaphthalene 57 and 2-bromo-3-chloronaphthalene 56 with a half-life of 48 minutes at 40°C.

Using dimethyl sulfoxide as solvent six products were obtained:
45 59.2% 2-chloronaphthalene; 62 5.5% C_{11}H_{9}ClSO; 63 0.4% C_{11}H_{9}ClSO;
57 30.9% 2,3-dichloronaphthalene; and 56 4.1% 2-bromo-3-chloro-
naphthalene.

Using d₆-dimethyl sulfoxide as solvent the same six products were obtained. Deuterium incorporation and isolated yields were:

33.3% 2-chloronaphthalene 45, of which 11.4% was nondeuterated,
30.4% was monodeuterated, 50.2% was di(deuterated and 8.1% was trideuterated; 1.4% C₁₁H₉ClSO 62 (deuterium incorporation not determined); 0.6% C₁₁H₉ClSO 63 with four deuterium atoms; 13.1%
2,3-dichloronaphthalene 57 of which 16.4% was nondeuterated,
73.1% was monodeuterated and 10.1% was di(deuterated; and 1.9%
2-bromo-3-chloronaphthalene 56 of which 55.8% was nondeuterated and 44.2% was monodeuterated. Overall recovery of material accounted for 50.3% of the starting material.

Using tetrahydrofuran three products were obtained:
19.9% 2-chloronaphthalene 45; 26.0% 2,3-dichloronaphthalene 57;
and 54.1% 2-bromo-3-chloronaphthalene 56.

**Reaction of 2,3-dichloronaphthalene 57 and 2-bromo-3-chloronaphthalene 56 with potassium t-butoxide:** In a similar way, the 1:19 mixture of 57 and 56 was reacted with potassium t-butoxide in dimethyl sulfoxide. Gas chromatography indicated that the dihalonaphthalenes were totally consumed, producing solely 2-chloronaphthalene 45. In d₆-dimethyl sulfoxide 1.5% was nondeuterated, 40.2% was monodeuterated, 38.9% was di(deuterated and 19.4% was trideuterated. No reaction occurred when this mixture was reacted with potassium t-butoxide in tetrahydrofuran.

**Reaction of 2-chloronaphthalene 45 with potassium t-butoxide:** The
reaction of 2-chloronaphthalene \(^{45}\) with potassium \(^{t}\)-butoxide in \(d_6\)-dimethyl sulfoxide as described above yielded 59.2\% nondeuterated 30.5\% monodeuterated and 10.3\% dideuterated 2-chloronaphthalene \(^{45}\).

**Reaction of 33 with lithium diethylamide:** A 100 ml three-necked flask was fitted with a mechanical stirrer, an addition funnel and a rubber septum and cooled to \(-15^\circ C\). In a nitrogen environment the lithium diethylamide (18 mmole) was prepared in situ by the dropwise addition of a solution of 2 ml of diethylamine in 9 ml of dry ether to 1.28 ml of 90\% \(n\)-butyl lithium in hexane in 15 ml of dry ether. After stirring for 15 minutes, 0.500 gm (1.8 mmole) of 33 dissolved in 10 ml of dry ether was added dropwise over thirty minutes. The reaction was allowed to warm to room temperature and to stir for one hour. It was poured into ice-water and extracted into ether. The ethereal layer was washed with saturated ammonium chloride solution, saturated sodium chloride solution and then dried with magnesium sulfate. After filtration of the drying agent and removal of the solvent, the residue was separated first on a silica gel preparative thin layer chromatography plate and then on a 6 ft x 1/2 in. 20\% SE-30 on Chromasorb P column. Five products 17.3\% 2-chloronaphthalene \(^{45}\), 3.7\% \(^{64}\) base peak m/e 184; 19.5\% \(^{65}\) base peak m/e 218; 34.6\% \(^{66}\) base peak m/e 218; and 24.8\% \(^{67}\) base peak m/e 218 were detected.

**Sodium amide reduction of bromobenzene 4:** Sodium amide was prepared in situ. Ammonia was condensed into a 1-liter three-necked flask fitted with a dry ice-acetone condenser, an Herschberg stirrer and an addition funnel. Upon addition of a small piece of sodium, the solution
immediately turned blue. Ferric nitrate hydrate (0.3 gm) was added and stirring continued until the blue color dissipated and the black precipitated catalyst had formed. The remainder of the sodium (6.9 gm; 0.3 mole) was added in small pieces and the solution allowed to stir (approximately 2-1/2 hours) until all the blue color dissipated leaving a dark brown solution. The bromobenzene \( \mathcal{X} \) (47.07 gm; 0.3 mole) was added dropwise over a twenty minute period. After stirring an additional twenty minutes, 105 gm (1.96 moles) of ammonium chloride was added, followed by approximately 200 ml of dry ether. After evaporation of the ammonia, water was added to dissolve the salts and the ethereal layer separated from the aqueous layer. The ethereal layer was washed with acid to remove the aniline \( \mathcal{Z} \), then dried with sodium sulfate and carefully distilled. In this way 0.2740 gm of benzene \( \mathcal{I} \) was obtained and 23.669 gm of bromobenzene \( \mathcal{X} \) recovered. The benzene \( \mathcal{I} \) was further purified and identified by gas chromatography and by its ultraviolet spectrum. The aqueous layer was made basic with solid sodium hydroxide and the aniline \( \mathcal{Z} \) extracted into ether. After drying, the ethereal layer was distilled affording 2.3962 gm of aniline \( \mathcal{Z} \).
REFERENCES


55. Private communication.
APPENDIX I

Spectra
APPENDIX II

Pyridoxal Catalyzed Synthesis of Tryptophan

in Model Enzyme Systems
ABSTRACT

Pyridoxal Catalyzed Synthesis of Tryptophan
in Model Enzyme Systems

Dorothy Wolf

As an extension of the investigations into Snell's proposed intermediates in pyridoxal catalyzed reactions, tryptophan was synthesized through the condensation of indole with 1) serine and 2) pyruvate and ammonia in the presence of pyridoxal and zinc(II) or aluminum(III). The serine system produced PMR and paper chromatographically detectable quantities of tryptophan in the pH 5-7 range following heating of the above described solutions to 75-80°C for one hour at reagent concentrations of 0.4M serine: 0.5M indole: 0.2M pyridoxal: 0.1M aluminum(III). Under similar conditions reagent concentrations of 2M pyruvate: 2M ammonia: 0.5M indole: 0.2M pyridoxal: 0.1M metal (II or III) were necessary in order to detect tryptophan. Separation difficulties precluded isolation and yield determinations.

Several questions were raised about the validity of Snell's mechanism as the result of some experiments and alternate schemes were therefore investigated. The serine-zinc(II) system never yielded tryptophan, whereas the serine-aluminum(III) and both pyruvate-ammonia systems did. Since Metzler and Snell previously established that zinc(II) does not break serine down to pyruvate and ammonia, whereas aluminum(III) does, doubt was shed on Snell's scheme, especially in
relation to the $\alpha$-aminoacrylic acid pyridoxlidene metal complex intermediate. As a further complication the pyruvate-ammonia systems gave an oxidation product of 3-indolepyruvic acid (a red pigment) as a major byproduct. Bearing these seeming discrepancies in mind two alternate schemes were proposed. The first was ruled out due to the offending intermediate being common to both it and Snell's mechanism. The second was ruled out by the failure to obtain tryptophan via the transamination of 3-indolepyruvic acid. PMR studies were carried out on all possible combinations of reagents not previously investigated throughout the pH 1-13 range to determine what intermediates were present. The importance of pyridoxal was established by the lack of reaction for all systems not containing pyridoxal and for all systems substituting salicylaldehyde for pyridoxal. In the final analysis Snell's mechanism can explain all the data obtained.
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INTRODUCTION

Shortly after the first isolation of tryptophan by Hopkins and Cole\textsuperscript{1} from trypsin digested casein, the dietary necessity of this amino acid was established.\textsuperscript{2} Continued interest in diseases associated with tryptophan deficiencies, especially in some Third World countries\textsuperscript{3} and renewed interest in tryptophan metabolic disorders\textsuperscript{4} emphasize the importance of finding a commercially acceptable synthesis of tryptophan and of better elucidating the mechanisms involved in the biosynthetic pathways to and from tryptophan. Of special interest is the necessity of the vitamin B\textsubscript{6}, pyridoxal, as a cofactor in many of the anabolic and catabolic reactions of tryptophan. Much success has been achieved in studying these reactions through the use of model enzyme systems.

Synthetically tryptophan was long considered one of the difficultly accessible compounds. Figures 1 and 2 summarize successful laboratory syntheses of tryptophan investigated or improved upon since approximately 1901. Historically the first attempts were based on the condensation of 3-indolealdehyde with hippuric acid. Overall yield of d,l-tryptophan based on indole using the 3-indolealdehyde route, was improved from Ellinger and Flamand's\textsuperscript{5} 3.5% yield in the first successful synthesis to Elks, Elliot and Hems'\textsuperscript{6} 27% yield. Limitations on this route were primarily the low yield synthesis of 3-indolealdehyde and this first step was therefore the primary focus of early experimentation. Nevertheless, the yield of this step remained no higher than 40%
obtained in the Grignard reaction of 3-indolemagnesium iodide with ethyl formate.\textsuperscript{9}

The discovery of the Mannich reaction in 1937 stimulated an upsurge in tryptophan synthesis research, this time utilizing Mannich bases especially that derived from gramine. This research resulted in several successful laboratory syntheses, in particular Albertson and Tullar's\textsuperscript{36} Mannich type condensation of gramine with ethyl-α-cyano-α-acetamidoacetate, followed by alkaline hydrolysis to give tryptophan in 71\% yield based on indole. Butenandt and Hellmann\textsuperscript{29} also obtained a 71\% yield of tryptophan by condensing piperidylmethylformamidomalonic ester with indole, again in a Mannich fashion. Included in Figure 2 are Fischer indole based syntheses, not necessarily passing through gramine. Of these the highest overall yield was obtained by Liang et al.\textsuperscript{32} through the condensation of diethylacetamido-n-propionalmalonate with phenyl hydrazine in the presence of triethylamine followed by cyclization using the Fischer indole synthesis. Saponification, decarboxylation and deacetylation of the resulting diethyl-acetamido-(3-indolylmethyl)-malonate yielded tryptophan in 70\% yield based on the starting malonic ester.\textsuperscript{11}

It can easily be seen that although these syntheses provide a relatively high yield route to tryptophan, the large number of steps and intermediate isolations make them commercially non-competitive with the current bacterial production of tryptophan. Two recent investigations provided ideas for a possible commercial synthesis of tryptophan. First, Snyder and MacDonald\textsuperscript{37} condensed indole with α-acetamidoacrylic acid in the presence of acetic acid and acetic anhydride to give a 57.7\% yield of acetyltrypophan. Second, the reversibility of tryptophanase
Fig. 3. Snell's Mechanism

\[ \text{Aldimine} \]

\[ \text{Racemization} \quad \text{Transamination} \]

\[ \text{Ketimine} \]

\[ \text{Condensation} \quad \text{Elimination} \]

\[ \alpha'-\text{Aminoacrylic Acid Derivative} \]

\[ \text{Elimination} \quad \text{Condensation} \]

\[ \text{Ketimine} \]

\[ \text{Transamination} \quad \text{Racemization} \]

\[ \text{Aldimine} \]
yielding a bioassayable quantity of tryptophan from ammonium ion, indole and pyruvate was demonstrated by Watanabe and Snell.\cite{38} Bearing in mind Snell's proposed mechanism of the pyridoxal-metal ion model enzyme system (Fig. 3) and the limited success of Metzler, Ikawa and Snell\cite{39} synthesizing tryptophan from serine and indole in this model enzyme system, the investigations discussed in this thesis into the reactions of serine or pyruvate and ammonia with indole in the presence of pyridoxal and zinc(II) or aluminum(III) lend additional support to this mechanism especially since tryptophan was synthesized. Had the yield been reasonable this may have provided an inexpensive commercial route to tryptophan.

The imine intermediate of Snell's mechanism for the model enzyme system provides a common intermediate that can explain the various reactions catalyzed by pyridoxal - racemization, transamination, elimination, condensation and decarboxylation. Tautomerization of the aldimine changing the $\alpha$-carbon of the amino acid from $sp^3$ to $sp^2$ hybridization destroys the assymetry of this carbon atom. Hydrolysis of this tautomer results in transamination. Racemization is the result of hydrolysis of the aldimine following equilibration of the aldimine and ketimine tautomeres.\cite{40} Baddiley\cite{41} proposed an alternate mechanism,
necessitating the bis complex for the transamination of tyrosine and pyridoxal in the presence of nickel(II). Electron-sink properties of the pyridoxal, especially due to the large planar conjugated system, as well as the cationic metal, facilitate elimination from the α-carbon of the amino acid to yield the α-aminoacrylic acid derivative. Attack of the α-aminoacrylic acid derivative on a nucleophilic reagent results in condensation. Decarboxylation is hypothesized as the result of electronic rearrangement in the absence of metal ions of a six-membered cyclic transition state.

The importance of the imine intermediate has led to considerable study of its structure and formation. Kinetic studies of the closely related semicarbazone formation have shown sharp pH-rate profiles at slightly acidic hydrogen ion concentrations. Conant and Bartlett demonstrated general acid catalysis for this system. In neutral and alkaline solutions the rate-limiting step is the dehydration of the carbinolamine, whereas attack of the free nitrogen base on the carbonyl forming the carbinolamine is rate-limiting in acidic solution.
From these kinetic studies Anderson and Jencks developed a scheme for imine formation.

\[
\begin{align*}
R-NH_3^+ & \xrightleftharpoons[K_a]{\text{R-NH}_2} R-NH_2 + H^+ \\
R-NH_2 + R'-CHO & \xrightleftharpoons[K_e]{\text{R-NH-CHOH-R'}} R-NH-CHOH-R' \\
R-NH-CHOH-R' & \xrightleftharpoons[k_r(\text{H}^+)]{\text{R-N = CH-R'}}
\end{align*}
\]

Demonstration of the aldime-ketimine tautomerization is of equal importance to the proposed mechanism. Several groups claim to have isolated the two structures and established their identity through negative evidence - that is, the complexes were devoid of aldehyde and amine groups. Using proton magnetic resonance Gansow and Holm directly observed the conversion of aldime to ketimine complexes in the reaction of pyridoxamine with pyruvate in the presence of zinc(II) or aluminum(III). The hydrolysis of these complexes was not followed since formation of the alanine and pyridoxal from these reactants is well documented. Most recently Abbott and Martell detected nuclear magnetic resonance signals which they attribute to a resonance hybrid of the aldime and ketimine complexes.

Since the yield in any successful reaction is dependent on the concentrations of intermediates (excluding side reactions), maximization of the imine formation is imperative in this route to tryptophan. Alcoholic solutions greatly favor the formation of imine over aqueous solutions. However, the use of methanol or ethanol as solvent presents two problems. Unless perdeuterated alcohols are used solvent protons will give magnetic resonance signals obscuring the high
field region of the spectrum. Secondly, the metal ions necessary for catalysis and to inhibit the decarboxylation side reaction are only sparingly soluble in alcohol. Even in aqueous solution around neutral pH insoluble metal salts form precipitating most of the metal ions. The only extremely soluble metal complex at neutral pH observed so far is pyridoxalenephosphateglutaminato copper(II). Due to the use of PMR as a means of detection we are limited to diamagnetic ions. Nevertheless, the catalytically active species need not be in high concentration and may not even be the dominant species in solution.

Although tryptophan had already been detected using microbioassy techniques in the serine-indole system, the routine use of high resolution nuclear magnetic resonance now provides a tool for the rapid identification and yield determination of tryptophan both in the serine-indole and pyruvate-ammonia-indole systems. Besides this, the PMR can be used for the identification of intermediates and kinetic studies of the reaction. In order to use PMR in these types of systems, all possible combinations of reactants must first be studied with respect to pH, in order to identify their resonances and intermediates formed. Much of this preliminary work has already been realized. The previous PMR studies of pyruvate dimerization, pyridoxal, pyridoxamine, their imines formed with amino acids and their metal complexes as applied to the transamination and dehydration reactions sought to establish a correlation between chemical shifts of the various protons and acid-base equilibria of the compounds as determined by titrimetry. Ultraviolet spectroscopy had already been successfully correlated with solution equilibria.

Although many unexpected difficulties arose, the primary
objective of this investigation was achieved. Tryptophan was identified by proton magnetic resonance and paper chromatography in both the serine-indole and pyruvate-ammonia-indole systems. Isolation and yield determinations were not achieved due to the low yield of tryptophan caused by highly competitive side reactions. Investigations into the nature of the side reactions, in an attempt to minimize them, led to much additional information on the intermediates and metal catalysis involved in the application of Snell's mechanism to tryptophan syntheses. The use of this reaction for a commercial synthesis of tryptophan is not necessarily hopeless since no experiments were attempted using either an alcoholic solvent or metal ions other than zinc(II) or aluminum(III).
RESULTS

A. Pyruvate systems

1. Pyruvate: In aqueous solution in the pH range two to
   twelve three species of pyruvate exist, their relative concentrations
   varying with pH. At acidic pH the keto-form of pyruvic acid predominates
   with a chemical shift of approximately 70 hertz. The hydrated gem-diol
   also exists giving a broad resonance around 20 hertz, sharpening as
   the pH approaches neutrality to a resonance at 14 hertz. The width
   of this peak between pH two and four can be attributed to the overlap
   of the pyruvate gem-diol peak and the dimer methyl peak. That the
   dimer is present throughout the pH range is attested to by the
   resonance between 120 and 130 hertz due to the dimer methylene group.
   At pH 11.96 it is a resolved doublet at 118 hertz with J = 1.5 hertz.

   Tallman and Leussing\textsuperscript{50c} discussed base and metal ion
   catalysis of pyruvate dimerization. However, they did not mention
   observing dimer at acidic pH values. Considering the acid and base
   catalysis of aldol condensations, there is no reason not to expect
dimerization in acid solution. The general argument against acid
catalysis is that the rate of enolization is too slow. In this
system it is doubtful that this is the case, since we observed
perdeuteration attested to by lack of PMR signals other than HOD,
within five minutes of dissolving sodium pyruvate and adjusting the
pH in the range two to twelve when deuterium oxide was used as solvent.
Fig. 8. Chemical shift versus pH for the Pyruvate System

Chemical shift plots for different conditions.

- Pyruvate - Anion
- Pyruvate - Zinc
- Pyruvate - Aluminum
Although the relative concentrations were not determined, the extent of dimerization is definitely greater as the hydrogen ion concentration decreases.

2. Pyruvate-ammonia: In order to determine whether an imine is formed between pyruvate and ammonia in aqueous solution this system was studied with respect to pH. In studying the pyruvate-glycinate system, Leussing and Stanfield\textsuperscript{50a} observed three peaks which they assigned to the carbinolamine (one peak at approximately 0 hertz) and the cis and trans ketimines (two peaks in the range 39 to 64 hertz). In the pH range six to nine we observed a very low intensity peak.
between 29 and 31 hertz. Although this peak is 10 hertz upfield from the pyruvate-glycinate imine, it still may be the imine, especially since it was again observed in the pyruvate-ammonia-zinc(II) system. Slight downfield shifts in the pyruvate dimer methyl and methylene peaks were also noted over the free pyruvate system, possibly indicating an imine of the dimer. These same dimer changes were exaggerated when zinc(II) was added. Some structure in which a slight positive charge is carried by the methylene carbon would yield such a downfield shift. The inductive effect due to a six-centered structure in which a proton

![Chemical structure](image)

is shared between nitrogen and oxygen for the mixed pyruvate-pyruvate imine dimer would produce such a shift. Substitution of zinc(II) for the proton would produce a greater chemical shift.

3. Pyruvate-zinc(II): The changes in dominant solvated pyruvate species with respect to hydrogen ion concentration in the presence of zinc(II) are similar to but exaggerated over those for pyruvate alone. In acidic solution the dominant species is free pyruvic acid and in basic solution free pyruvate dimer. From pH four through pH seven pyruvate forms mono and bis complexes with zinc(II) probably as four and/or five membered chelate systems. These are observed in

![Chemical structures](images)
the PMR as peaks in the region of approximately 45 to 65 hertz. This upfield shift is unexpected since the inductive effect of the complexed zinc(II) should cause a shift in the opposite direction. The shift should be more pronounced for the five-membered chelate than the four-membered chelate since it is carried over one less carbon in the five-membered case. However, as Leussing and Stanfield\textsuperscript{50a} point out an upfield shift with ionization arises when no strong metal-ligand or hydrogen bond exists. That is, the metal-ligand bond for zinc(II) may be quite labile and therefore exert no strong inductive effect. One would expect a predominance of the five-membered chelate because the optimum between the opposing effects of entropy and strain energy favor five-membered systems over four- and six-membered ones. Complexation is further noted by a heavy white precipitate formed at pH 5.84 and persisting through pH 8.2. Leussing and Stanfield isolated and studied the precipitate to determine that it is the bis pyruvate zinc(II) complex, probably the zinc salt of the dimer.

4. Pyruvate-aluminum(III): A comparison of the pyruvate-zinc(II) and pyruvate-aluminum(III) spectra demonstrate pyruvate-metal complexation over a far wider pH range in the aluminum(III) case. At pH approximately twelve the metal complex is still the dominant species in solution over the dimer. No methylene dimer peaks are observed until pH 8.2. The inductive effect of the aluminum ion is far greater than the zinc ion as evidenced by the 20 hertz downfield shift of pyruvate protons. This also indicates that the aluminum complex is far less labile than the zinc complex, as has been noted for almost all aluminum complexes studied so far. Peaks between 20 and 60 hertz are possibly
due to dimer methyl protons shifted downfield due to complexation as five- and six-membered chelates with the aluminum(III). The seven-membered chelate is also possible, however as Tallman and Leussing point out it is highly unfavored by entropy effects. If these species existed in solution, one would expect the methylene protons to be shifted by from 10 to 50 hertz placing them under the water peak and its spinning side bands.

5. Pyruvate-ammonia-zinc(II): The graphical presentation of this system appears to be a composite of the pyruvate-ammonia and the pyruvate-zinc(II) systems. As in the pyruvate-ammonia system a peak appears at 29 hertz, however a full pH unit before the pyruvate-ammonia
system. A second peak appearing over the same pH span at 54 hertz could be due either to a shift caused by metal complexing of a pyruvate imine or it could be due to a metal complex of pyruvate as discussed in the pyruvate-zinc(II) section. A few observations that indicate the former is the correct interpretation are that 1) at pH 4.17 the solution turned distinctly yellow, an indication that imine is forming; and 2) at pH 6.98 precipitation of a metal complex occurred, a full pH unit after the bis pyruvate-zinc(II) complex precipitates. Peak areas in the pH region seven to twelve were distinctly different from either the pyruvate-zinc(II) or the pyruvate-ammonia systems.

B. Pyridoxal-ammonia systems

Of primary interest in studying possible intermediates in this reaction is whether an imine forms between pyridoxal and ammonia. Regardless of the PMR spectra obtained a strong indication of imine formation is precipitation observed at pH 5.0 in the pyridoxal-ammonia system. Pyridoxal alone forms no such precipitate at any hydrogen ion concentration. The presence of a metal ion seems to stabilize this imine formed, since with 2:2:1 pyridoxal:ammonia:zinc(II) the precipitation is delayed by a full pH unit. When aluminum is used no precipitation is observed.

A comparison of the graphical presentation of pyridoxal with the pyridoxal-ammonia systems shows the changes expected. The greatest differences are multiple 4-CH peaks and an additional peak in the 260 to 270 hertz region. This could be attributed to a downfield shift of the 5-CH$_2$OH peaks due to a through-space effect of the positive charge on the nitrogen in a carbinolamine. Inductive effects would not
apply strongly since bond polarization would have to be transmitted through four bonds. One would expect very little difference in the 2-CH₃ peak, since it is quite removed from the reactive center.

C. Salicylaldehyde

In an attempt to avoid the expense of using pyridoxal in the decomposition of serine, and/or the synthesis of tryptophan, salicylaldehyde was used to mimic the pyridoxal. A system, 0.4M serine, 0.8M indole and 0.4M zinc(II) at pH 5.5, which was known to produce pyruvate and was hoped to produce tryptophan when pyridoxal is present was used. Aliquots taken at times zero, one hour and twenty-four hours gave no indication of pyruvate or tryptophan in the PMR. Overnight the solution had however deposited white needles. These were isolated by suction filtration, washed and dried. They dissolved completely in acidic solution giving an PMR of salicylaldehyde and serine. Addition of ammonium sulfide gave no precipitate, indicating no metal complexation of the imine.

D. Red-pigment

The solubility behavior of the red compound obtained by the oxidation of the product of the reaction between indole and pyruvate at pH 6.5 in the presence of zinc(II) or aluminum(III) tended to indicate
that it might be the red pigment of Krishnamurth, Buckley and Duerre.\textsuperscript{55} The similarity in the paper chromatographed $R_f$ value of 0.76 compared with their 0.71 lends support to this identification. The similarity of the PMR of the red pigment in water to the aqueous PMR of 3-indolepyruvic acid indicates the red pigment may be Krishnamurth, Buckley and Duerre's hypothesized oxidation product. Indirectly this identification would indicate that 3-indolepyruvic acid is either an intermediate in the synthesis of tryptophan from indole, pyruvate and ammonia, or that it is a byproduct. Since no pyridoxal was used in this synthesis the compound obtained can not be 3-indolylidene-3-indolyl-4-(2-methyl-3-hydroxy-5-hydroxymethylpyridyl)methane\textsuperscript{56} the other red pigment observed in these types of systems.

![Image of molecular structure](image1)

![Image of molecular structure](image2)

E. Serine decomposition

The use of PMR to study the kinetics of serine decomposition to pyruvate severely limited the scope of the investigation, although this appeared to be an easy, rapid method of detecting pyruvate.
Fig. 6. Serine Decomposition in the Pyridoxal–Zinc(II) System
Precipitation at all hydrogen ion concentrations was so great that the aluminum(III) system could not be studied at all. For the zinc(II) system precipitation became prohibitive above pH 5.5. As indicated on the following graph, no dramatic change (one and one half to three hours) in the half-time of the reaction was noted over a thirty-five fold decrease in hydrogen ion concentration. The white precipitate was not identified. However, from Leussing and Stanfield's\textsuperscript{50a} work, the indications would be strong that it is the pyruvate-dimer zinc(II) complex. If this were the case these kinetic studies would be completely invalid, since some precipitate was present in all aliquots, and only solvated species contribute to sharp PMR signals. The synthesis of the white precipitate was elusive on a scale large enough for its identification. The sharp drop-offs of pyruvate concentration after the maximum is reached at approximately four and a half hours tends to indicate that pyruvate is rapidly removed from solution, possibly as the dimer-zinc(II) complex. Some indication that a free radical mechanism might be involved appeared when all solutions for time zero exposed to light indicated pyruvate present. When we noted this, the experiments were repeated with care taken to keep them in the dark throughout the reaction and PMR spectroscopy. The importance of light was not investigated here. However, in the tryptophan syntheses no changes were noted when the reaction was carried out in the dark or under a sunlamp.

F. Tryptophan syntheses

Tryptophan was detected both by PMR and paper chromatography in the 4:4:2:1 serine:indole:pyridoxal:aluminum(III) and the
20:20:4:2:1 pyruvate:ammonia:indole:pyridoxal:zinc(II) or aluminum(III) systems. It was not detected in the 4:4:2:1 serine:indole:pyridoxal:zinc(II), 4:20:4:2:1 pyruvate:ammonia:indole:pyridoxal:zinc(II) or aluminum(III) nor in the transamination of 3-indolepyruvic acid in the presence of pyridoxal and either zinc(II) or aluminum(III) (4:20:2:1 3-indolepyruvic acid:ammonia:pyridoxal:metal ion). All solutions containing ammonia were taken to strongly basic pH to simplify the spectra. Those containing serine were taken to strongly acid pH. The spectra of commercial tryptophan, pyridoxal and zinc(II) at pH less than two and pH greater than twelve are given for comparison.

A comparison of the several systems at pH 5.5 produced a maximum yield of tryptophan in the ammonia-pyruvate-indole-pyridoxal-aluminum(III) system. This system was then used to obtain a pH profile of the reaction. Using paper chromatography in which a known volume of known concentration was chromatographed, the naked eye could easily detect differences in the intensity of the ninhydrin produced purple spot. For this system tryptophan is produced only in the pH range five to seven with a maximum between six and six and a half. All of the systems were repeated in this pH range. No qualitative differences were noted over the previous results.

In view of the possibility that a free radical mechanism might be active, the series of experiments for all systems at pH 6.0 to 6.5 were repeated under a sunlamp and in the dark. No differences were noted. Reaction is precluded in the absence of pyridoxal in all cases.

In all reaction systems the white precipitate formed and had to be removed before PMR spectra were taken. For paper chromatograms,
the reaction mixture had to be acidified and extracted into n-butanol. Chromatograms gave ultraviolet and Ehrlich detectable spots at Rf values approximately 0.90, 0.75(red pigment), 0.65, 0.53, 0.45 (tryptophan), 0.35 and 0.26.

The yellow color which rapidly became red-brown on heating to 75-80°C under a nitrogen atmosphere is very likely one of the two red pigments. The optimum reaction time appears to be ninety minutes.

Isolation of the tryptophan was attempted using several methods.

Yield determinations were not possible since no system was found whereby the tryptophan could be isolated from all reactants and byproducts. This should however be possible, since paper chromatography easily separated the tryptophan from all other materials. In the PMR integration could not be achieved for several reasons: 1) the phase control around the water peak was so poor that at the amplitude necessary to see the tryptophan aromatic, methylene and methine peaks no steady baseline could be obtained; 2) higher concentrations of
reactants and/or products yielded severe precipitation, obscuring all peaks in the baseline; and 3) overlap between the pyridoxal 4-CH, 6-H and tryptophan aromatic and between the tryptophan methylene and water and its spinning side bands resulted in not being able to distinguish integration due to the tryptophan from other materials. Generally the methine peak was so small it was unobservable.
DISCUSSION

The detection of tryptophan in any of these reaction systems was in itself an achievement and reinforced the ideas in Snell's mechanism for pyridoxal catalysis. However, on closer inspection the lack of tryptophan in the serine-indole-pyridoxal-zinc(II) system and the production of tryptophan in both aluminum(III) systems and in the pyruvate-ammonia-indole-pyridoxal-zinc(II) system shed doubt on the mechanism. It is well established that aluminum(III) catalyzes the decomposition of serine to pyruvate and ammonia, whereas zinc(II) does not. Thus, it appeared that the presence of pyruvate and ammonia preceded the synthesis of tryptophan. For this reason two alternate mechanisms were proposed.

Alternate mechanism I (Fig. 9) is doubtful for two reasons. First, as Tallman and Leussing point out the five-membered chelate structure is favored over the four-membered structure. The stability of the pyruvate-metal complex is at least partially due to chelation since basicity alone cannot explain the stability. The strain in the four-membered complex would counteract the stability introduced by chelation. This problem is avoided in the five-membered chelate. To further support the five-membered ring Tallman and Leussing conclude that for the dimer, the five-membered ring predominates over the six-, seven- or eight-membered structures. For this reason the formation of \( \alpha \) and \( \beta \) is doubtful. Secondly, the intermediates \( \xi \) and \( \eta \) differ

-110-
Fig. 9. Alternate Mechanism I
from  in Snell's mechanism only by possessing the hydroxy group on
the al-carbon rather than the  carbon. In this alternate mechanism
the pyridoxlidene imine in no way facilitates the dehydration to
yield the al-aminoacrylic acid pyridoxal aldimine. The dehydration
is a reaction totally divorced from the rest of the system in this
mechanism. Differences in the two mechanisms yielding reaction would have to occur before intermediate \(_1\) because it is Snell's intermediate.

The second alternate mechanism was definitely ruled out since no tryptophan was obtained in the attempted transamination of 3-indolepyruvic acid. This is possibly due to the lack of tautomerization of the pyruvate imine as observed by Leussing and Stanfield.\(^{50a}\)

In one respect the lack of tryptophan in the transamination of 3-indolepyruvic acid was a benefit, since a double isotopic labelling competitive experiment would have been necessary to distinguish Snell's mechanism from alternative II. Perdeuteration of pyruvate in the

\[
\text{Excess} \quad \text{CH}_3\text{C} = \text{COOH}
\]

presence of zinc(II) or aluminum(III) in deuterium oxide and the synthesis of the red pigment from indole and pyruvate in the presence
Fig. 10. Alternate Mechanism II

\[ \text{CH}_2\text{O} + \text{M}^{n+} \rightleftharpoons \text{HOCH}_2\text{CH} = \text{OH} \rightleftharpoons \text{HOCH}_2\text{CH} = \text{N} \rightleftharpoons \text{HOCH}_2\text{CH} = \text{O} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{M}^{n+} \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{N}_2 \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{NH}_4^+ \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{NH}_4^+ \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{NH}_4^+ \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{NH}_4^+ \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]

\[ \text{HOCH}_2\text{C}=\text{NH} + \text{NH}_4^+ \rightleftharpoons \text{HOCH}_2\text{C}=\text{NH} \rightleftharpoons \text{HOCH}_2\text{C}=\text{OH} \]
of zinc(II) and aluminum(III) demonstrates that intermediate 6 exists in these solutions. This gives one explanation for the byproduct 3-indolepyruvic acid.

Investigations into the importance of a free radical mechanism were prompted by the reported synthesis of tryptophan from 3-indolepyruvic acid and ammonium hydroxide in aqueous solution when irradiated with bactericidal ultraviolet light. For this reason all experiments were conducted both in the dark and under an ultraviolet lamp. No differences were noted, ruling out this possibility.

After the discrediting of these three alternative mechanisms, Snell's was reexamined. The results obtained in these experiments can be explained by Snell's mechanism if one concludes that zinc(II) is not strongly cationic enough to facilitate the dehydration of serine to the \(\alpha\)-aminoacrylic acid pyridoxlidene zinc(II) intermediate. Aluminum with the greater charge does facilitate this dehydration. In the pyruvate system, the \(\alpha\)-aminoacrylic acid derivative is arrived at by loss of a proton from the \(\beta\)-carbon. Since the ligand is essentially becoming anionic it binds the metal more tightly and any cationic species facilitates this reaction. A second source of the byproduct
3-indolepyruvic acid is the hydrolysis of intermediate before tautomerization.

From the results of the various systems of possible intermediates, it appears that in the pyruvate-ammonia systems, pyridoxal is the carrier of ammonia by binding it as the imine. The metal serves as a template on which the transamination with serine or pyruvate takes place, followed by dehydration and condensation with indole. Whether the transamination is essentially an internal or an external reaction can only be determined by kinetic studies of the reaction.\(^{57}\)

Several things must be borne in mind when the intermediates involved are considered. One is that PMR, without computer of average transients or Fourier transforms is relatively insensitive \((0.01M)\) and slow on most reaction time scales. That is, the catalytic specie is often not in high concentration and can be rapidly exchanging with solvent or in equilibrium with another structure thereby broadening the intermediate signals into the baseline. Therefore, intermediates not at all detected in the pyruvate and pyridoxal systems might be involved in the reactions.

Another important consideration is that precipitation occurred in almost all solutions. That is the concentrations initially were not those pertaining at the time of reaction. Not only were metal and pyruvate (as the dimer) removed from solution, but pyridoxal was yielded inactive and indole removed from solution by reaction to form the red pigments.

The most severe limitation in this investigation was however, the inability to isolate tryptophan from the reaction mixtures. Ion exchange resins presented two difficulties. One is that indole
containing compounds are rapidly degraded in the presence of such resins. Secondly, the elution of tryptophan by pH occurred when the complex with the metal and pyridoxal is the dominant species. Even when the metal was removed by precipitation with ammonium sulfide the tryptophan-pyridoxalidene imine eluted as a single compound. The charcoal adsorption chromatography gave only a small yield when a known amount of tryptophan was chromatographed. The successful separation on paper was supposed to be mimicked by silica gel and Hyflo-Supercel. This, however, proved untrue. No separation between the 3-indolepyruvic acid red pigment and tryptophan could be achieved.

Nevertheless, tryptophan was successfully synthesized in several systems. Highest yield was obtained by reacting 20:20:4:2:1 pyruvate:ammonia:indole:pyridoxal:aluminum(III) adjusted to pH 6.2 in an inert atmosphere one hour at 75-80°C. Although the yields were low, and the tryptophan could not be isolated, the use of methanol or ethanol as solvent might increase the yield and facilitate isolation, making this still a possible commercial synthesis of tryptophan.

The series of experiments necessary to understand this reaction support Snell's mechanism and demonstrate that the limitation in the decomposition of serine in the presence of pyridoxal and zinc(II) is due to the lack of dehydration to the α-aminoacrylic acid derivative. If it could be demonstrated that the mechanisms in the model enzyme system also occur in vivo this would likely be the most well understood biological reaction system and might contribute to an understanding and control of the diseases - cancer and mental illnesses - associated with tryptophan excesses.
EXPERIMENTAL

Pyridoxal monohydrochloride, serine, tryptophan, indole and 3-indolepyruvic acid were obtained from Sigma Chemical Company and used without further purification. Dimer-free sodium pyruvate was also obtained from Sigma Chemical Company and PMR showed that it was sufficiently dimer-free to proceed without purification. In one case reagent grade salicylaldehyde served to mimic pyridoxal.

Reagent grade zinc and aluminum sulfates, and ammonium chloride were used to make stock solutions of these chemicals. For qualitative experiments in aqueous systems, pH adjustment was achieved using various arbitrary concentrations of reagent grade hydrochloric acid and sodium hydroxide. In the series of quantitative experiments the weight of added known concentrations of hydrochloric acid and sodium hydroxide were used to determine the molality of the final reaction mixtures.

Some experiments were carried out in 99.8% deuterium oxide obtained from Columbia Organic Chemicals, in which case pD was adjusted using $d_2$-sulfuric acid obtained from Stohler Isotope Chemicals and using NaOD obtained by dissolving clean sodium in deuterium oxide. pD was corrected to pH using the relationship $pD = pH + 0.40$. Stock metal solutions for these experiments were made by dissolving zinc oxide in acidic $D_2O$ and anhydrous aluminum chloride in $D_2O$. 

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Physical Methods

Hydrogen ion concentrations were determined at room temperature using a Corning model 5 pH meter equipped with a glass electrode. Proton magnetic resonance spectra were recorded at a probe temperature of 40°C on a Varian A-56/60 spectrometer. Chemical shifts were measured to approximately ±1 hertz relative to an internal standard of 1% v/v t-butanol. The kinetic study to determine the rate of decomposition of serine to pyruvate was carried out on a Bruker HFX-10 spectrometer. Pyruvate concentration was determined by evaluating the integral of the pyruvate signal at 96 hertz relative to both the 2-CH₃ (at approximately 100 hertz) and the 6-H signal (at approximately 550 hertz) of pyridoxal. (The 2-CH₃ and 6-H signals vary with pH.)

Detection Methods

A. Paper chromatography: In order to rapidly determine whether tryptophan had been formed in the several reaction mixtures, paper chromatography was employed. The reaction mixture was acidified to pH less than two and extracted repeatedly with ether to remove any excess indole, 3-indolepyruvic acid and pyruvic acid. The aqueous layer was then extracted into n-butanol saturated with 0.6N hydrochloric acid. The n-butanol solution was spotted onto Whatman No. 1 filter paper and developed using a 4:1:5 v/v/v n-butanol:acetic acid:water solvent system. Compounds containing the indole ring system were located using a hand-held ultraviolet lamp and amino acids were located using ninhydrin. Rf values proved to be the same as those measured by Krishnamurth, Buckley and Duerre.

B. PMR sample preparation: When PMR was used to detect
trypophan in the reaction mixtures, the spectra were simplified by adjusting the pH such that virtually no metal-aldimine complex was present, thus leaving only peaks due to species free in solution. Generally, this was accomplished by adjusting the pH to less than two. However, in the ammonia systems the ammonium protons fall in the tryptophan aromatic region in strongly acidic media. In strongly basic media (pH ~ 12) the rate of exchange of the ammonium protons is great enough on the PMR time-scale that they are broadened into the baseline. Again, the reactants are substantially free in solution at pH twelve. For this reason all systems containing ammonia were adjusted to pH ~ 12 before recording PMR spectra. In some cases precipitate remained after adjusting the pH to either a strongly acidic or strongly basic value. These solutions were filtered either through glass wool or sintered glass before recording the spectra.

Separation Techniques

Several techniques of column chromatography were attempted to isolate the tryptophan obtained in the various reaction mixtures. Ion exchange chromatography using Amberlite IRA-400 (strong base) and Dowex 50W-X8 (strong acid) proved untenable, since elution position is determined by pH. In both cases the tryptophan-pyridoxal aldime metal complex came off as the complex. Also, with indole ring containing compounds contact with ion exchange resins and mechanical desalters should be avoided due to the ease of degradation of these substances.

Two types of adsorption chromatography were attempted with limited success. Activated charcoal separated the tryptophan from the
reactants, however only in very small yields. A 50:50 w/w mixture of silica gel and Hyflo-Supercel was used to mimic the successful separation on paper.

The adsorbent was slurried in distilled water and washed with water saturated with n-butanol; n-butanol saturated with water and finally with 60:15:25 v/v/v n-butanol:acetic acid:water. The column was poured into this solvent system and the reaction mixture partitioned with the same. The tryptophan was successfully isolated from a model reaction mixture, however the byproduct 3-indolepyruvic acid present in all actual reaction mixtures eluted simultaneously with the tryptophan on this column with this solvent system.

**Reaction Systems**

A. Pyruvate systems: [Pyruvate, pyruvate-ammonia, pyruvate-zinc(II), pyruvate-aluminum(III), pyruvate-ammonia-zinc(II) and pyruvate-ammonia-aluminum(III)] Pipetted quantities of the appropriate stock solutions were placed in a 5 ml volumetric flask and distilled water added to make the solution 0.2M in all reagents. A concentrated sodium hydroxide solution was used to adjust the pH of the solutions to approximately half integral values in the pH range two to twelve. PMR spectra were run immediately. In some instances of heavy precipitation the solutions were filtered before obtaining a spectrum.

B. Pyridoxal-ammonia systems: [Pyridoxal-ammonia, pyridoxal-ammonia-zinc(II), pyridoxal-ammonia-aluminum(III)] The above solutions were made to 0.2M in pyridoxal and ammonia, and 0.1M in metal ion in D₂O from pipetted quantities of the appropriate stock solutions. Again the pH was adjusted to half integral values over the pH range two to
twelve, however NaOD and/or D$_2$SO$_4$ solutions were used for the adjustment. Some solutions required filtration before PMR spectra were recorded.

C. Serine-indole-salicylaldehyde: The hydrogen ion concentration of a solution of 2.1021 gm (0.4M) serine, 5.7519 gm (0.4M) ZnSO$_4$·7 H$_2$O and 2.25 ml (0.4M) salicylaldehyde in 50 ml of water was adjusted with sodium hydroxide solution to pH 5.5. To this solution 4.6914 gm (0.8M) cf indole was added. The reaction was allowed to proceed at 120°C under reflux in a nitrogen atmosphere. Aliquots were withdrawn at one hour and at twenty-one hours, acidified to pH less than two and filtered through glass wool into PMR tubes. Overnight the remaining reaction solution, allowed to stand at room temperature, deposited needle-like white crystals of serinesalicylaldimine. The crystals were collected by suction filtration, dried and redissolved in a strongly acidic medium. Treatment of this solution with ammonium sulfide showed that zinc was not complexed with the aldime.

D. Synthesis of the Red Pigment: Ten milliliters of a reaction solution was made 2M in pyruvate and 0.1M in aluminum(III) or zinc(II). The pH was adjusted to 6.5. The solution was added to 2.3428 gm (2M) of indole and reacted four hours at 70-75°C under an inert atmosphere. The reaction was cooled in an ice bath and acidified to approximately pH two. After stirring open to the air for two days the solution was a red color containing a definite red precipitate. The reaction solution was further acidified and repeatedly extracted with ether. The product was extracted from the combined ether extracts into 0.1N NaOH. Impurities were removed from the combined sodium hydroxide
extracts with more ether. The sodium hydroxide solution was then acidified, first turning milky white and finally into a brown tar. The brown tar dissolved completely in ether and was separated from the aqueous layer. Paper chromatography of a sample of the ether solution on Whatman No. 1 filter paper using 4:1:5 v/v/v n-butanol:acetic acid: water as the developing solvent yielded one red-orange spot at Rf 0.76. The bulk of the ether solution was dried over anhydrous magnesium sulfate and the ether was removed on a rotary evaporator. The dark residue was redissolved in 0.1N NaOH and slowly acidified to the milky white stage. The white precipitate was collected by suction filtration and dried. On standing in air the precipitate again turned a red-brown color.

E. Serine decomposition: Reaction solutions were made in ten milliliter volumetric flasks to 0.1M:0.1M:0.05M from pipetted quantities of serine, pyridoxal and zinc(II) or aluminum(III) stock solutions respectively. One milliliter of 10% t-butanol in water was added as internal standard and reference. The pH was then adjusted using sodium hydroxide solutions, withdrawing aliquots into a PMR tube at each half integral value over the pH range two to twelve. The PMR tubes were placed in a 70°C constant temperature bath for periods of 5, 10, 30, 60, 120, 240 and 480 minutes. The reactions were quenched in ice water and stored in the freezer until just prior to recording the spectra at room temperature on the Bruker HFX-10.

Tryptophan Syntheses

A. Serine-indole-pyridoxal-zinc(II) or aluminum(III):
Distilled water was added to pipetted quantities of serine, pyridoxal and zinc(II) or aluminum(III) stock solutions to make five milliliters
of reaction solution approximately 0.4M:0.2M:0.1M. The pH was adjusted using known concentrations of sodium hydroxide solution so that the molality could be determined. Finally, 0.2930 gm of indole was placed in a round bottom flask and the reaction solution added. The reaction was carried out with magnetic stirring under an inert atmosphere at 75-80°C for various lengths of time ranging from ten minutes to twenty-four hours. Optimum reaction time was found to be ninety minutes.

B. Pyruvate-ammonia-indole-pyridoxal-zinc(II) or aluminum(III): Two sets of experiments were carried out using these systems. Again, pipetted quantities of pyruvate, ammonia, pyridoxal and zinc(II) or aluminum(III) stock solutions were combined and diluted to yield 5 ml of, in the one case, 0.4M:2M:0.2M:0.1M reaction solution and in the other case, 2M:2M:0.2M:0.1M reaction solution. The hydrogen ion concentration was adjusted and the molality determined. The reaction solution was then added to 0.2930 gm of indole, in the first case, and 0.2344 gm of indole in the later case, in a round bottom flask. The reaction was allowed to proceed under the conditions described for the serine-indole-pyridoxal systems above.

C. 3-Indolepyruvic acid-ammonia-pyridoxal-zinc(II) or aluminum(III): Stock solutions of ammonia, pyridoxal and zinc(II) or aluminum(III) were pipetted, mixed and diluted to 2M:0.2M:0.1M in five milliliters. Sodium hydroxide solutions were used to adjust the pH and the final molality was determined. The reaction solution was added to 0.4064 gm of 3-indolepyruvic acid and the reaction carried out as described above.

All of the above tryptophan syntheses were also carried out omitting the pyridoxal. A third series of experiments used the same
reaction conditions plus a sunlamp to investigate the importance of
a possible free radical mechanism.
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